THE SEXUAL DEVELOPMENT

OF THE COMMON MARMOSET MONKEY,

CALLITHRIX JACCHUS JACCHUS

by

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the degree of
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To Angela

In Nature's book of infinite secrecy
A little can I read.

WILLIAM SHAKESPEARE

Antony and Cleopatra (c. 1606)

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Abbott D.H. (in press) The physical, hormonal and behavioural development of the common marmoset, Callithrix jacchus jacchus, in The Biology of the Callitrichidae (ed. by Rothe H., Wolters H.J. and Hearn J.P.) University of Göttingen.

Abbott D.H. and Hearn J.P. (1978) Physical, hormonal and behavioural aspects of sexual development in the marmoset monkey, Callithrix jacchus. J. Reprod. Fert., 53, 155-166.

Abbott D.H. and Hearn J.P. (1979) The effects of neonatal exposure to testosterone on the development of behaviour in female marmoset monkeys, in Sex, Hormones and Behaviour (Ciba Symposium (New Series) No. 62) pp. 000-000 Excerpta Medica, Elsevier North-Holland Press; Amsterdam.

Hearn J.P., Abbott D.H., Chambers P.L., Hodges J.K. and Lunn S.F. (1978) Use of the common marmoset, Callithrix jacchus, in reproductive research, in Marmosets in Experimental Medicine (ed. by Gengozian N. and Dienhardt F.W.), Primates in Medicine, Vol. 10, pp. 40-49. Karger, Basle.

ABSTRACT OF THESIS

This is a study of sexual development and differentiation in the marmoset monkey, Callithrix jacchus jacchus. The effects of perinatal steroid treatments on the anatomy, physiology and behaviour of females of various mammalian species are reviewed. A summary of the literature on marmoset development is included.

Detailed measurements of growth, plasma sex hormone levels and reproductive behaviour were obtained from captive marmosets at birth until 600-1000 days of age. Body weights and knee-to-heel lengths were similar for both sexes. Males had high levels of testosterone from 5-100 days of age and testosterone began to rise again, coincident with the pubertal growth of the testis, at about 250 days. Pre-ovulatory levels of oestradiol were found in females of 200 days old and over, but ovulations, as indicated by high progesterone levels ($\geq$ 20 ng/ml), did not occur for nearly another 200 days. The majority of males and females were copulating by 400-500 days old. Males of this age could ejaculate spermatozoa and females were able to conceive. However, first conceptions occurred more frequently when males and females were 500-600 days of age. Maternal age and experience was found more important than paternal for rearing offspring.

In all captive groups only the dominant female reproduced. In families, sexual behaviour was virtually limited to the parents, but their maturing daughters ovulated and their sons could ejaculate spermatozoa. In newly-formed peer groups of unrelated animals, clear rank orders were achieved by fighting. The dominant male and female ranked over all the others, formed a pair bond, and attempted to prevent any other sexual relationship. Older and heavier males held high rank,

but no such correlation was found with females. Subordinate females copulated but stopped ovulating. There was some evidence of accompanying elevated prolactin levels. The fertility of subordinate males was less affected because they copulated and could ejaculate spermatozoa. The mechanisms of reproductive inhibition and their possible roles in the marmoset's monogamous social system are discussed.

Neonatally androgenized female marmosets displayed enhanced rough-and-tumble play and masculine sexual behaviour, but without the loss of feminine behaviour. There was no effect on aggression or fertility, but the clitoris of each animal was permanently hypertrophied. Other primates, such as the rhesus monkey and the human, may have a period of neonatal behavioural differentiation, but the function of high testosterone levels in newborn males remains to be determined. The differentiation of marmoset behaviour after birth may partly explain why females born co-twin to males are chimeric, but are otherwise normal.

CHAPTER 1 INTRODUCTION AND THE REVIEW OF LITERATURE

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1.1 Introduction

The sexual development of mammals may be divided into two stages : sex determination, whereby the genetic sex of an individual dictates whether an ovary or testis develops; and sexual differentiation, in which testicular hormones control the development of the brain and peripheral tissues, which otherwise would mainly develop as female. This thesis is concerned with hormonally induced sexual differentiation and the maturation of this process in a primate. Figure 1.1 summarizes the sequence of events during sexual development, and the influence of hormonal sex on phenotypic and brain sex will now be reviewed.

1.2 The differentiation of male and female

1.2a The internal and external genitalia

It was recognised early in the history of endocrinology that blood-borne substances influence the development of the reproductive system (Keller and Tandler, 1916; Lillie, 1916; 1917). Since that time hundreds of research papers have described hormonally-induced sexual differentiation and the conditions under which such an influence takes place (see reviews by Jost, Vigier, Prepin and Perchellet, 1973; Price, Zaaijer, Ortiz and Brinkmann, 1975). We now know that before the foetus of either sex differentiates, it possesses both Wolffian and Müllerian ducts, a urogenital sinus and a primordial genital tubercle. In males the testicular hormones cause the development of the Wolffian ducts and the accessory glands derived from the urogenital sinus (e.g. the prostate), the growth of the genital tubercle into a penis, and the regression of the Müllerian ducts.

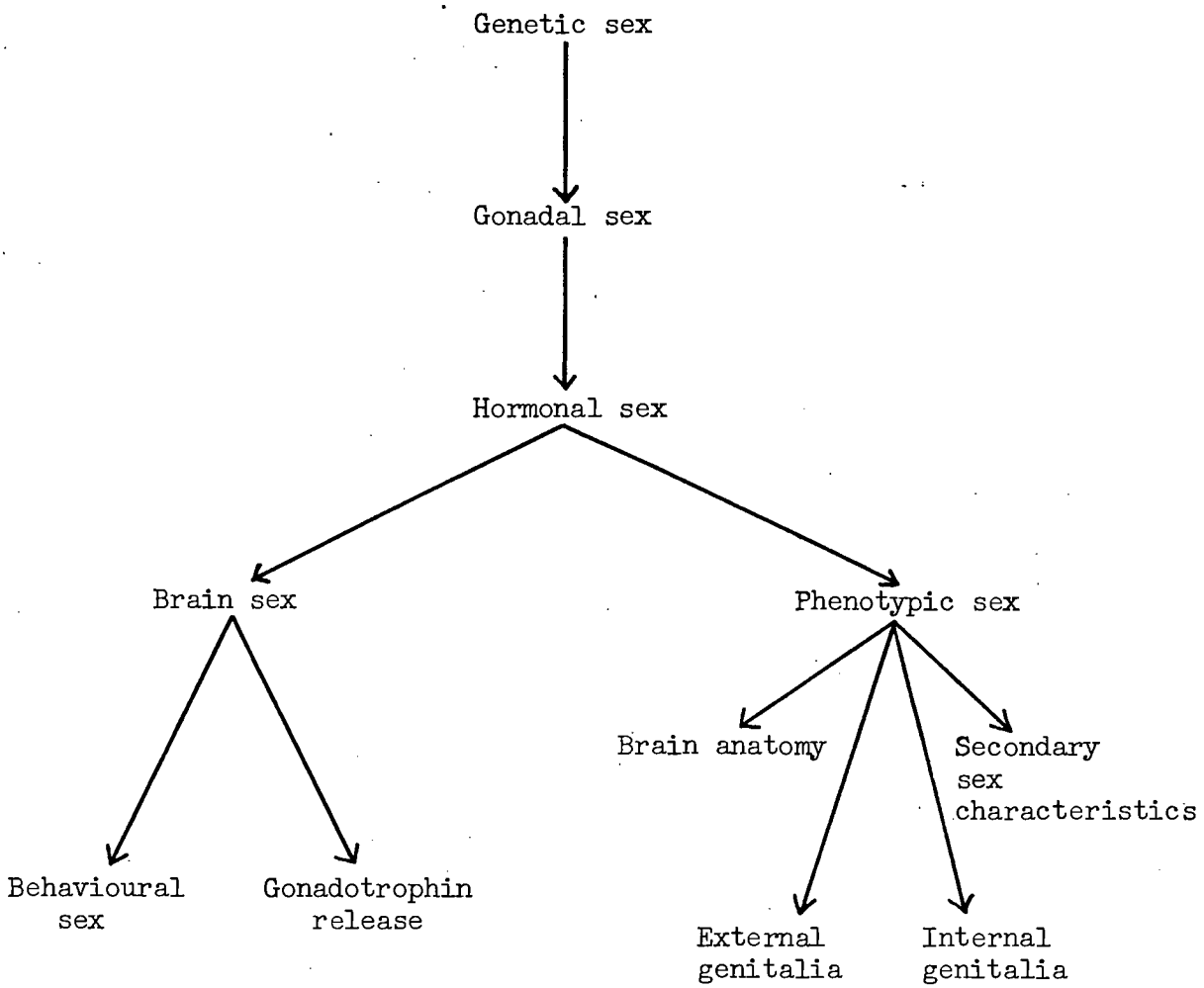

FIGURE 1.1: The sequence of events during sexual development

(from Clarke, 1976)

All,but the latter,can be mimicked in female foetuses by giving androgens to their mothers during critical stages of gestation. This has been repeated in many species including rats (Greene, Burrell and Ivy, 1939), mice (Turner, Haffen and Struett, 1939), hamsters (Bruner and Witschi, 1946), rabbits (Jost, 1947), guinea pigs (Phoenix, Goy, Gerall and Young, 1959), cows (Jost, Chodkiewicz and Mauléon, 1963), dogs (Beach and Kuehn, 1970), sheep (Short, 1974a; Clarke, Scaramuzzi and Short, 1976), rhesus monkeys (van Wagenen and Hamilton, 1943) and humans (Wilkins, Jones, Holman and Stempfel, 1959; Wilkins, 1960). Müllerian duct regression is caused by another testicular secretion (Jost, 1947, 1953; Josso, 1974; Blanchard and Josso, 1974; Josso, Picard and Tran, 1977). In the absence of testicular hormones differentiation is essentially female: the Wolffian ducts regress, the Müllerian ducts develop into the Fallopian tubes and the uterus, which in turn interacts with the urogenital sinus to form the vagina, and the genital tubercle becomes a clitoris. However there is growing evidence, in rats and mice, that complete female differentiation may also require the active influence of ovarian hormones and not just the lack of testicular ones (Shapiro, Goldman and Root, 1974; Attardi, Geller, Ohno, 1976; Döhler, in press; Döhler and Hancke, 1978).

Freemartins in cattle provide a classical example of abnormal sex differentiation in mammals (see review by Marcum, 1974). Freemartins are genetic females which, in twin or multiple pregnancies share a common placental circulation with at least one male twin or triplet etc. (Jost et al, 1973). These females are chimeras, possessing XY cells that originate from the male co-twin via the vascular connection (Ohno, Trujillo, Stenius, Christian and Teplitz, 1962;

Short, Smith, Mann, Evans, Hallett, Fryer and Hamerton, 1969; Ohno, 1969; McLaren, 1976 review). Their ovaries are stunted and sterile, and frequently contain sterile seminiferous tubules after the animals are born. The derivatives of the Müllerian ducts are more or less completely absent, and some parts of the Wolffian ducts are frequently present (seminal vesicles, vasa deferentia and epididymes) (Lillie, 1916, 1917; Gerneke, 1967; Short et al, 1969; Jost, Vigier and Prepin, 1972; Jost et al, 1973; Jost, Perchellet, Prepin and Vigier, 1975). The external genitalia of freemartins remain essentially feminine, but may be partially virilized and a prostate gland may develop from the urogenital sinus (Jost et al, 1975).

The basic defect in freemartinism is therefore partial sex reversal of the gonads. The development of the bovine freemartin starts to depart from the normal female pattern about the 48th day of gestation when the ovaries suddenly stop growing (Jost et al, 1972, 1973). This is also the time when the external genitalia of the male foetus begin to show the first signs of masculinization, indicating that the foetal testes have commenced androgen secretion. Lillie (1916, 1917) and Keller and Tandler (1916) originally proposed that sex hormones from the male co-twin were responsible for the female's freemartin condition, but this theory is now discounted (if male 'sex hormones' are taken to mean only androgens). Testosterone or other androgens administered to pregnant cows completely masculinized the external genitalia of their female offspring, but did not affect the internal genitalia or the ovaries (Jost et al, 1963; Jainudeen and Hafez, 1965). Hence, while androgens alone could not duplicate the freemartin syndrome, other testicular secretions could not be eliminated.

On day 51 of gestation, the uterus and Fallopian tubes in the freemartin suddenly start to regress (Jost et al, 1972) inferring that some other male-derived factor was at work. As day 51 is also the time when the Müllerian duct derivatives of the male foetus start to regress, the Müllerian duct inhibiting hormone secreted from the male's testes (Josso, 1974; Josso and Blanchard, 1974) was implicated as the causal agent. Jost et al, 1972 have advanced the concept that this hormone inhibits both the ovary and the Müllerian ducts and that would explain the correlation between their degrees of inhibition. The first signs of masculinization of the freemartin are seen about day 60 of gestation, long after masculinization is first observed in the normal male (Jost et al, 1972, 1973). Seminiferous tubules begin to appear in the freemartin's gonads, transforming them into very small testes. Oddly enough, foetal testes have a pronounced local masculinizing action, exerting their greatest effects on the immediately adjacent structures. For example, asymmetry of the reproductive tract occurs in foetuses with only one functional testis, such as in hermaphroditic or intersex individuals, because female duct development (such as the Fallopian tube and uterus) predominates on the side away from this testis (e.g. goat: Short, Hamerton, Grieves and Pollard, 1968; pig: Breeuwsma, 1970; human: van Niekerk, 1974). It is possible that the testicular tissue in the freemartin's gonads could produce sufficient androgen to result in local, internal masculinization, but perhaps insufficient to bring about extensive masculinization of the external genitalia. Testosterone from a male co-twin is unlikely to reach a female co-twin because of placental aromatization of androgens (Pierrepoint, Stewart and Rack, 1969). Certainly the freemartin's

gonads are capable of secreting appreciable quantities of testosterone after birth (Short et al, 1969). Hence, although the freemartin may be masculinized by her own androgens, rather than those of her male twin, there is as yet no explanation for the belated development of seminiferous tubules in her ovaries. XX/XY chimeric bulls do not escape unscathed from this intra-uterine experience either, since they may lapse into premature sterility soon after puberty (Stafford, 1972).

Another theory of freemartinism was that XY cells from the male foetus may have promoted testicular tissue formation in the female co-twin's gonad (Fechheimer, Herschler and Gilmore, 1963). This theory was elegantly castigated when ligation of the vascular link between male and female calf foetuses was found to prevent freemartinism, while chimeric cells were found in the female's liver (Vigier, Locatelli, Prepin, de Mesouil, du Brisson and Jost, 1976). Some factor from the male foetus had been prevented from reaching the female and XY cells, themselves, or their components (e.g. the male-determined H-Y antigen as proposed by Wachtel, Ohno, Koo and Boyse, 1975 and Ohno, Christian, Wachtel and Koo, 1976) were not capable of inducing the freemartin syndrome. Witschi (1965; 1970) proposed that gonadal development was under the control of testicular inductor substances (as well as inhibitors), and the sex reversed gonads of the freemartin may be a result of an inducer passing from the male co-twin to the female causing retention of medullary cords (precursors of seminiferous tubules) in the female. However, there are several arguments against this. These include the rare occurrence of true hermaphrodites exhibiting bilateral asymmetry of the gonads with the presence of a

testis on one side and an ovary on the other (Short, 1970; van Niekerk, 1974) and the normal development of XX/XY chimeric female marmoset monkeys (Benirschke and Brownhill, 1962). Nevertheless, without some inductor substance leaking across the placental circulation from the male's testes, the presence of seminiferous tubules in the freemartin's gonad is hard to explain. In the case of marmosets, twins are the rule (see below), and in almost every case large vascular anastomoses develop between the two placental circulations at a very early stage of gestation (Wislocki, 1939). Heterosexual twins therefore show XX/XY leucocyte chimerism, yet the freemartin condition never occurs. Therefore, before it is possible to establish conclusively the identity of the agent or agents that are responsible for the freemartin syndrome, it appears that an explanation for the absence of freemartinism in the marmoset is essential (see Chapter 7 for further discussion).

The reason why freemartins are comparatively rare in sheep, goats and possibly pigs is because placental vascular anastomoses rarely occur even though there is frequently fusion of the placental membranes (Marcum, 1974; Short, 1974b). In the horse (Vandeplassche, Podliachouk and Beaud, 1970) and the human (Race and Sanger, 1975) blood chimerism has been demonstrated in twin pregnancies, although there is no evidence of any freemartin effect and individuals have been reported as fertile. However, in both cases, vascular anastomoses may develop relatively late in pregnancy, long after sexual differentiation was complete. Nevertheless, in the human, people who have developed as spontaneous XX/XY primary chimeras often show ambiguous external genitalia or other anatomical evidence of their intersexual status (e.g. an enlarged clitoris and an ovotestis: Gartler, Waxman

and Giblett, 1962; de la Chapelle, Schröder, Rantanen, Thomasson, Niemi, Tülikainen, Sanger and Robson, 1974). Primary chimeras are individuals formed by the aggregation or combining of cells from different embryos at a very early stage of development, or spontaneously arising at fertilization if more than one spermatozoon fertilizes a single egg, or an egg and its polar body (McLaren, 1976).

In summary, regardless of dose and duration of androgen treatment, no-one has succeeded in inducing complete sex reversal in mammals. While androgen treatment early in the life of genetic females can completely masculinize their external genitalia, it does not halt the differentiation of the developing ovary or internal feminine genitalia and it does not stimulate testicular development. Some other factor(s) are at work.

1.2b The brain mechanisms regulating gonadal function

1.2b(i) Mammals other than primates

The differentiation of mechanisms regulating gonadal function (excluding the initial determination of ovaries or testes) also occurs in early life, but later than the differentiation of the genitalia. This is assumed because androgens only masculinize genetic females prenatally in animals with relatively long gestation periods, such as guinea pigs (Phoenix et al, 1959), dogs (Beach and Kuehn, 1970), and sheep (Clarke, 1976; Clarke, Scaramuzzi and Short, 1977). In species with relatively short gestation periods, such as rats (Harris, 1964), mice (Barraclough and Leathem, 1954) and hamsters (Swanson and Brayshaw, 1973), androgens were only effective if given postnatally. Such androgenized females were rendered anovulatory in later life. However, the ovulatory capacity of their ovaries was not impaired by

the androgen treatment because ovaries transplanted from androgen sterilized animals to normal females commenced cyclic activity and ovaries transplanted from normal females to androgen sterilized rats became acyclic (Bradbury, 1941; Harris and Levine, 1965). In fact the sterility of these androgenized rats was attributed to altered hypothalamic control of gonadotrophin release (secreted from the pituitary) from the 'cyclic' pattern of the female to a 'tonic' pattern similar to the male (Barraclough and Gorski, 1961). Seemingly, androgenization prevented these females from releasing an ovulatory discharge of gonadotrophin in response to oestrogen feedback (Brown-Grant, 1974a). Normal female rats exhibit this positive feedback response, whereas normal males do not (Davidson, 1974).

In androgenized female sheep, (rendered partially or sometimes totally anovulatory by exposure to testosterone during different stages of gestation for different lengths of time: (Short, 1974a; Clarke, Scaramuzzi and Short, 1977)),the anovulatory condition was due to a reduction in hypothalamic sensitivity to oestrogen (Clarke and Scaramuzzi, 1978) rather than to masculinization and therefore differed from the situation in rats where perinatal androgenization of females abolished positive feedback (Brown-Grant, 1974a,b).Apparently sheep are intermediate between rats and monkeys with respect to brain mechanisms that regulate positive feedback and ovulation because early androgen treatment abolished positive feedback in female rats, had a partial effect in ewes and was ineffective in monkeys (see section 1.2b(ii)).

Depending on the circumstances, gonadal steroid hormones regulate pituitary gonadotrophin secretion by inhibitory or stimulating mechanisms. The concept of inhibitory or negative feedback (controlling follicle

stimulating hormone (FSH) and luteinizing hormone (LH) secretion : gonadotrophins from the pituitary) was postulated independently in 1932 by Moore and Price, and Hohlweg and Junkmann when they found a simple inverse relationship between circulating gonadal steroid concentration and pituitary gonadotrophin secretion (see Hisaw, 1947; van Rees, 1964; Labhsetwar, 1973, for reviews). Everett, Sawyer and Markee (1949) suggested that oestrogens acting on the central nervous system initiate the ovulatory release of LH through a stimulatory or a positive feedback mechanism (controlling cyclic release of LH) after Hohlweg (1934) and Everett (1948) found that administered oestrogens caused or advanced ovulation in female rats. This was later confirmed in rats byFerin, Tempone, Zimmering and Vande Wiele (1969), Gorski,(1971), Caligaris, Astrada and Taleisnik (1971), and in sheep by Goding, Catt, Brown, Kaltenbach, Cumming and Mole (1969), Short (1974a), Karsch and Foster (1975), and Clarke, Scaramuzzi and Short (1976).

Pfieffer (1936) was the first to suggest that testicular hormones caused masculinization of these mechanisms controlling gonadotrophin secretion. In his classical experiment he transplanted rat gonads between the sexes soon after birth and at puberty introduced ovarian tissues into the anterior chambers of their eyes to observe any evidence of cyclicity. In males or females castrated at birth and given ovarian transplants, the ovarian tissue transplanted to the eyes after puberty showed cyclic activity. In entire males, or males and females castrated at birth and given testicular transplants, the ovarian implants in the eyes did not show cyclic activity. Pfieffer (1936) concluded that neonatal testicular secretions caused the anovulatory syndrome by masculinizing the rat's pituitary gland and

thereby altering gonadotrophin secretion. This conclusion was not surprising as workers at that time had linked only LH and FSH secreted from the pituitary gland with gonadal function (Fichera, 1905; Smith, 1926, 1927; Smith and Engle, 1927; Fevold, Hisaw and Leonard, 1931). It was not until 1955, following the suggestion by Marshall (1936) that the anterior pituitary might be under the control of substances manufactured in the brain and Popa and Fielding's (1930, 1933) description of a system of portal vessels connecting the hypothalamus with the anterior pituitary, that Harris (1955) presented the first convincing evidence for the neurochemical control of the anterior pituitary via the portal vessels. The conclusions of his epic monograph were swiftly confirmed by Porter and Jones a year later and by the detection of a luteinising hormone-releasing factor in 1960 (McCann, Taleisnik and Friedman). Eventually, by 1961, Barraclough and Gorski had conclusively linked the sterile androgenization syndrome in female rats with their impaired hypothalamic function.

However, recent findings in rhesus monkeys suggest that Pfieffer's (1936) original conclusion might be correct (see Section 1.2bii). Oestrogen might be able to exert both its negative and positive feedback actions on gonadotrophin secretion at the level of the pituitary gland. Stimulatory effects of oestrogen on gonadotrophin secretion have been demonstrated at the hypophysical level in the rat (Greeley, Allen and Mahesh, 1975; Drouin, Lagace and Labrie, 1976) but it is unlikely that the pituitary is the primary target for oestradiol in the initiation of the pre-ovulatory gonadotrophin surge in this species (Sarkar, Chiappa and Fink, 1976; Goodman, 1978).

The impaired hypothalamic function of the androgenized female rats is not identical to that of males. Plasma LH and FSH levels are

similar in androgen sterilized and normal female rats (Mallampati and Johnson, 1973), whereas FSH (Johnson, 1971), but not LH (Brown-Grant, 1973; Mallampati and Johnson, 1973) is elevated in normal males. Following castration the plasma LH and FSH levels in androgenized and normal female rats are lower than in castrated males (Brown-Grant, 1974c). Furthermore, when androgenized females are castrated in adulthood, the rise in plasma gonadotrophin levels is similar to the female response, occurring more slowly than the rise following castration of males (Davidson, 1974). These data suggest that androgenization does not produce a masculine form of tonic secretion.

Paradoxically, neonatal administration of oestrogen was found to be more effective than androgen in inducing sterility in genetically female rats (Turner, 1941; Hale, 1944; Gorski, 1963; Harris and Levine, 1965; Brown-Grant, 1974b). It was subsequently suggested that 'androgenization' of the hypothalamic centres controlling gonadotrophin release was caused by aromatization of androgens to oestrogens within the brain cells (Reddy, Naftolin and Ryan, 1974). This claim was supported by the greater aromatizing capacity of hypothalamic tissue in neonatal male rats compared with females (Reddy et al, 1974), the failure of a non-aromatizable androgen, dihydrotestosterone, to induce the anovulatory condition in female rats (Whalen, and Luttge, 1971; McDonald and Doughty, 1972; Korenbrot, Paup and Gorski, 1975), and the binding of radioactively aromatized testosterone to the oestrogen receptors of only the hypothalamic/amygdaloid region of the brain (McEwen, Lieberburg, Maclusky and Plapinger, 1976; Westley and Salamann, 1977). While Naftolin and his co-workers (Naftolin, Ryan, Davies, Reddy, Flores, Petro, Kuhn, White,

Takoaka and Wolin, 1975) have re-emphasised that androgens are active in brain tissue via their conversion to oestrogen, the reason for this process is not clear. However, several authors have postulated an explanation (Plapinger and McEwen, 1973; Plapinger, McEwen and Clemens, 1973; Doughty, Booth, MacDonald and Parrott, 1975a; McEwen, et al, 1976). They report the presence of an extracellular protein (fetoneonatal oestrogen binding protein or alpha-fetoprotein) in foetal and neonatal rats that binds oestrogens but not androgens. Consequently these proteins would allow androgens to reach the brain cells of males, and 'masculinize' the tissue, but would protect females against their own or their mother's circulating oestrogens. Nevertheless, Shapiro, Goldman, Bongiovanni and Marino (1976) have suggested that the elevated progesterone levels of females protects them from the masculinizing actions of their endogenous oestrogens and androgens, as progesterone is known to antagonise the effects of both these hormones (Dorfmann, 1967). What mechanism is more effective remains to be seen; perhaps the high progesterone in females actively imprints female sexual differentiation (Shapiro et al, 1974, 1976).

Androgens and oestrogen antagonists have been used with mixed success in preventing anovulatory sterility in female rats (Doughty et al, 1973; 1975b; Brown-Grant, 1974b; Gottlieb, Gerall and Thiel, 1974; Hayashi, 1974). The ambiguous results reflect the lack of knowledge of their mechanisms of action.

Flerkó (1968; 1975) has proposed that the sterility of androgenized females is caused by the inactivation of the "oestrogen sensitive trigger mechanism" responsible for initiating ovulatory surges of gonadotrophin release in females. Norepinehrine (NE) is believed to be one of the neurotransmitters which acts as a messenger

between the oestrogen sensitive areas in the hypothalamus and the neurons containing gonadotrophin releasing hormones (Flerkó, 1975). An intraventricular injection of NE was effective in causing ovulation in androgenized females (Tima and Flerkó, 1974), suggesting that the functional cause of sterility could be a failure of neurosecretory cells to release an ovulatory quota of gonadotrophin releasing hormone because of a lack of stimulation via neurotransmitters. The induction of ovulation in androgenized female rats by administering LH-RH supports this suggestion (Borvendeg,Herman and Bajusz, 1972; Hahn, Lai and Greenslade, 1974; Hahn and McGuire, 1978). Flerkó (1975) proposed that the latter hormone is not produced to a sufficient extent in androgenized females because androgenization significantly reduces the number of oestrogen-responsive receptors in the hypothalamus.

In support of this hypothesis a number of authors had found that oestrogen was taken up by the hypothalamus at lower rates in androgenized females than in normal females (Flerkó, Mess and Illei-Donhoffer, 1969; McGuire and Lisk, 1969; Maurer and Woolley, 1971; Vertes and King, 1971). On the other hand, others had found no difference (Green, Luttge and Whalen, 1969; Whalen and Luttge, 1970; Attardi and Ohno, 1976; Maurer and Woolley, 1974). Thus the evidence that males and females differ in their ability to concentrate oestrogen in the hypothalamus or the brain in general is not convincing (Whalen, 1974). Of course, it is possible that during differentiation, hormones act on the brain not to alter biochemical mechanisms, but to alter growth and thereby neural connections. Raisman and Field (1971) have reported different synaptic connections in the pre-optic area of male and female rats after sexual differentiation and Salaman and Birkett (1974) have found that DNA, RNA and possibly

protein synthesis are necessary for the masculinization of the rat hypothalamus to occur (see also Gorski, 1973). In this respect the process is closely analogous to hormone-induced differentiation in other tissues of the body, for example in the mammary gland (Turkington and Kadohama, 1972) or the erythropoietic system (Harrison, Conkie and Paul, 1973).

Further evidence for morphological effects of oestradiol and testosterone on neuronal tissue stems from experiments on mouse hypothalamic and pre-optic tissue in vitro (Toran-Allerand, 1976, in press). The response of the cultured tissue to the addition of oestradiol or testosterone was most striking in the pre-optic area and the infundibular/premamillary regions and was characterised by extensive neuritic proliferation and the formation of dense plexuses of neural processes which extended far into the distal outgrowths. In fact, the gender of the adult rat can be determined by looking at thionin-stained 60 mμ sections of the medial preoptic area with the naked eye (Gorski, Gordon, Shryne and Southam (in press) and Gorski, Harlan and Christensen (in press) cited by Toran-Allerand, in press). The volume of this region is much larger in the male.

The above studies have shown that sexual differentiation of the mechanisms controlling steroid facilitation of LH secretion (the major requisite for ovulation) occurs during a restricted perinatal period (depending on the particular species used). After this period, during which androgen (or oestrogen) can exert organisational effects on the control of LH secretion, the former hormones can no longer have such an effect (Barraclough, 1961; Gorski, 1968; Lobl and Gorski, 1974). Androgens and oestrogens may activate the controlling neural mechanisms, but the functional organisation of the latter is fixed. The distinction between the activational and organisational effects of steroids

(Pheonix et al, 1959) has been central to the current understanding of sexual differentiation of neuroendocrine functions. However, two studies have suggested that the presence of the ovary during pre- or post-natal life can modify the effect of perinatal androgen on the control of LH secretion (Kikuyama and Kawashima, 1966; Arai, 1971). These researchers used the delayed anovulation syndrome (DAS) to study the role of ovarian secretions in the adult female rat. In this syndrome, female rats given a low dose of androgen neonatally show oestrus cycles after puberty, but become persistently oestrus and have polyfollicular ovaries at an early age (Gorski, 1968). The development of anovulation in these DAS animals is delayed if the animals are ovariectomized at an early post-pubertal age and implanted with ovarian grafts at a later age (Kikuyama and Kawashima, 1966; Arai, 1971). These data suggested that ovarian secretions in the adult animal may have organisational effects on the neural control of ovulation, and therefore the susceptible period for sexual differentiation of LH release might extend into adult life, at least after perinatal exposure to androgen. Harlan and Gorski (1978) recently confirmed these results but found no corresponding effects on behaviour. Whether this will be the case in other species remains to be seen.

Although considerable progress has been made to identify the way in which steroids modulate the activity of hormonal components within the brain which control reproductive processes (Kamberi, 1972; Ladosky and Wandscheer, 1975), the exact mechanisms operating in these systems are still not clear. However, in the non-primate female, the pattern of gonadotrophin release is cyclic whereas the male pattern is non-cyclic (Davidson, 1974). The basic sex difference is that females show an ovulatory discharge of gonadotrophin with appropriate steroid provocation (positive feedback) whereas males do not.

1.2b(ii) Primates (including man)

In comparison with rodents, little is known about the sexual differentiation of the neural mechanisms controlling gonadal function in primates.

The male foetus in the rhesus monkey (Resko, 1970, 1974, 1977) and in the human (Abramovich and Rowe, 1973; Reyes, Boroditsky, Winter and Faiman, 1974; Diez D'Aux and Murphy, 1974; Faiman, Winter and Reyes, 1976; Reyes, Winter and Faiman, 1976) experiences increased concentrations of plasma testosterone during early to mid-gestation, when sexual differentiation might be occuring. Their female counterparts show no increase (loc cit.), even though in the human FSH levels are clearly greater than in the male (Faiman, Reyes and Winter, 1974). The high testosterone concentrations in the male primate might influence brain development in a manner similar to that in rodents (section 1.2b(i)). The increase in testosterone concentrations apparently occurs in response to stimulation by chorionic gonadotrophin produced by the placenta (rhesus: Resko, 1977; human: Reyes et al, 1976). In the human, chorionic gonadotrophin (HCG) stimulated testosterone production in foetal testes (12-22 weeks of gestation) in vitro. However, the earliest demonstrations of an HCG receptor in the testes is at 26 weeks of gestation (Frowein and Engel, 1974). Human foetal pituitaries contain gonadotrophins (Levina, 1968) and synthesise FSH and LH in culture by 13 weeks of gestation (Groom, Groom, Cooke and Boyns, 1971) and hence may play a role in stimulating the testes.

There were no sex differences in the concentrations of circulating oestradiol levels in human (Reyes et al, 1976) and rhesus monkey foetuses (Resko, 1977). In the latter there were also no differences in circulating oestrone, androstenedione or dihydrotestosterone. However, female rhesus monkey foetuses did have higher

concentrations of plasma progesterone (Resko, 1977) suggesting that differentiation in primates might not be limited to the action of androgens. No such difference was found in the human, but the amounts of progesterone in the umbilical artery and vein did differ significantly according to the sex of the foetus (Hagemenas and Kittinger, 1973).

Newborn male rhesus and pig-tailed macaques (Robinson and Bridson, 1978) and humans (Forest, Cathiard and Bertrand, 1973a,b; Forest, Sizonenko, Cathiard and Bertrand, 1974b; Forest, 1975, in press) experience a further period of elevated testosterone levels not observed in females. In the macaques this lasts from about the first to the fourteenth week of life and in the human from the first to about the fifteenth week. Coincidentally, newborn human males also have higher levels of LH than females, but females have clearly higher FSH levels (Faiman, et al, 1974; Forest et al, 1974b). A similar gonadotrophin pattern is shown in the chimpanzee (Faiman et al, 1974). In both the macaques and the human there is no increase in oestradiol concentrations (macaques: Robinson and Bridson, 1978; human: Faiman et al, 1974, 1976; Forest, in press). Unfortunately, the effects of these neonatal hormonal fluctuations on the differentiation and/or maturation of the hypothalamic-pituitary-gonadal axis are unknown.

In accordance with the above findings, female primates were effectively masculinized with testosterone as fetuses (rhesus monkeys: van Wagenen and Hamilton, 1943; Dantchakoff, 1950; humans: Wilkins et al, 1955; Wilkins, 1960). However, in contrast to rodents and other non-primate species such as sheep, androgenization of the female foetus never induced the anovulatory syndrome in adulthood (rhesus monkeys: Wells and van Wagenen, 1954; Goy and Resko, 1972; Goy et al, 1977; Man: Wilkins et al, 1955, 1959; Wilkins, 1960). Yet

their external genitalia were as effectively masculinized as those of rodents or sheep.

Nevertheless, in both rhesus monkeys (Goy and Resko, 1972; Wilen, Goy, Resko and Naftolin, 1977) and the human (Jones and Verkauf, 1972) foetal androgenization delayed the menarche in these pseudo-hermaphrodites. Human true hermaphrodites have also been shown to ovulate (van Niekerk, 1974).

Female pseudo-hermaphrodites are genetically XX, possess ovaries and feminine internal genitalia, but have external genital structures that to some degree resemble those of a male. True hermaphrodites (as portrayed in a survey by van Niekerk, 1974) range genetically from XX, through a variety of XX/XY and XO/XY mosaics to XY, possess an ovary or ovo-testis on one side with an ovo-testis or testis on the other, a combination of masculine and feminine internal genitalia and their external genitalia also show a degree of masculinization. Ovarian function in female rhesus monkeys given either a large injection of testosterone (35 mg/kg.) at birth (Treloar, Wolf and Mayer, 1972) or regular injections of testosterone propionate (2 mg/kg.) between 6.5 and 14.5 months of age (Josylyn, 1973) was also unimpaired in adulthood. Similar results were found with girls given testosterone (40 µg - 1.5 mg) at birth to improve their chances of survival (Polishuk and Anteby, 1971). The only morphological affect reported was permanent clitoral hypertrophy in the latter rhesus monkey group.

Interestingly, female rhesus monkeys treated with testosterone propionate from infancy to menarche developed precocious menarche at one year of age (van Wagenen, 1949). However, puberty was reached at the same body weight as controls (the latter reached menarche at two

years). This is unlike neonatally androgenized female rats which developed precocious puberty at a lower mean body weight than controls (McDonald and Doughty, 1973).

Hence anatomical masculinization would appear to occur independently of hypothalamic sexual differentiation in the rhesus monkey and in Man. Consequently the ability of the hypothalamic-pituitary unit to discharge LH in response to oestrogen (i.e. show positive feedback) may not be a sexually dimorphic characteristic in primates. However, the data on this point are inconclusive. Initial attempts to obtain a female-like surge in castrated male rhesus monkeys were unsuccessful (Yamaji, Dierschke, Hotchkiss, Bhattacharya, Surve and Knobil, 1971) and only later, when LH levels in castrated animals were chronically suppressed with oestrogen before the oestrogen benzoate injection, was a positive feedback response obtained (Karsch, Dierschke and Knobil, 1973). Unfortunately, in the latter situation, the animals have been so far removed from their normal endocrine state that interpretation of the results is difficult. Positive feedback has yet to be demonstrated in intact male rhesus monkeys. It has been claimed that oestrogen may be capable of inducing LH release in normal (Kulin and Reiter, 1976) and homosexual men (Dörner, Rohde, Stahl, Krell and Masius, 1975), although the LH rises that were described are comparable in neither magnitude nor duration to those seen in normal women. A more detailed study by Van Look, Hunter, Corker and Baird,(1977a)provided no evidence for the presence of a positive feedback effect of oestrogen in either normal or hypogonadal men. Nevertheless a positive feedback response was demonstrated in a patient with XY pure gonadal dysgenesis (Van Look et al 1977a). Further evidence stems from a study of human males suffering

from the testicular feminization syndrome. These 'men' possess testes but develop phenotypically as females (Morris, 1953). Their intra-abdominal testes secrete normal amounts of testosterone (Jeffcoate, Brooks and Prunty, 1968), but all their tissues are insensitive to it. Yet such patients did not exhibit the positive feedback response to oestradiol administration characteristic of normal women (Van Look et al, 1977a; Aono, Miyake, Kinugase, Kurachi and Matsumoto, 1978), suggesting that the hypothalamus had been masculinized in spite of the androgen insensitivity. These patients have provided the first evidence for the lack of direct effects of androgen on the human hypothalamus, similar to the findings in rodents.

However, in contrast to this latter finding, female human pseudo-hermaphrodites have been found to ovulate in adulthood (Jones and Verkauf, 1971), indicating the existence of positive feedback. Furthermore, since ovulation has been well documented in many true hermaphrodites possessing an ovary or ovotestis on one side and an ovotestis or testis on the other, the presence of an androgen-producing gonad does not necessarily prevent the hypothalamus and pituitary from exhibiting positive feedback (Van Niekerk, 1974). Nonetheless, the dilemma of whether or not positive feedback is a sexually dimorphic characteristic in primates cannot be solved from studies on these naturally-occuring abnormalities until their early development is investigated and the exact malfunctions during development are identified.

The picture is confused even more by the recent findings of Hodges (1977) and Hodges and Hearn (1978) which have shown an oestrogen-induced LH response (positive feedback) in intact and castrated male marmoset monkeys, equivalent to that found in ovariectomized and intact

females. Whether such a response is unique to marmosets remains to be seen, and is discussed in Chapters 6 and 7.

The evidence for sexual differentiation of the neuronal mechanisms controlling gonadal function in primates would thus seem to vary between species. However, differentiation of these mechanisms in primates may not be solely the perogative of neuronal tissue. The recent findings of Nakai, Plant, Hess, Keogh and Knobil (1978) and Plant, Nakai, Belchetz, Keogh and Knobil (1978b)suggest that in the female rhesus monkey oestradiol can exert both a negative and positive feedback action on gonadotrophic secretion at the level of the pituitary gland. Hence the differences between males and females in their ability to exhibit positive feedback may also lie in this gland. Lesions had been previously created in the hypothalamus of the ovariectomized rhesus females to abolish endogenous LH-RH production, as evidenced by a profound reduction in gonadotrophin secretion (Plant, Krey, Moossy, McCormack, Hess and Knobil, 1978a). The latter results in no way exclude neural sites of oestradiol action in rhesus females with an intact hypothalamus, because Neill, Patton, Dailey, Tsou and Tindall (1977) have shown that LH surges were associated with elevations of LH-RH levels in pituitary portal blood. In non-primate species, such as the rat, the pituitary does not seem to have such a degree of autonomy (Sarkar et al, 1976; Goodman, 1978). At this stage one should be wary of equating primate sexual differentiation of the hypothalamus and pituitary with that of rodents (e.g. comparisons of rodent, primate and human data made by Dörner (1976)).

1.2c The brain centres controlling behaviour

Apart from masculinizing the anatomy and physiological processes of developing mammals, androgens can also cause permanent masculinization

of behavioural patterns. Females exposed to androgens during development provide an experimental model with which to examine the behavioural effects of these hormones.

Over 35 years ago Wilson, Young and Hamilton (1941) and others suggested that the development of behaviour might be under hormonal control (much of the work prior to 1959 is reviewed by Whalen, 1968). However, this line of research did not mature for another 20 years when Phoenix and his co-workers (1959) and Harris and Levine (1962) published their papers showing that testicular hormones, during the early stages of maturation, 'organise' or differentiate the tissues that mediate mating behaviour. Studies of the sexual behaviour of androgenized females have since been reviewed by Beach (1968; 1971; 1975), Whalen (1968; 1974), Phoenix, Goy and Resko (1968), Goy and Goldfoot (1973), Ward (1974) and Clarke (1976).

1.2c(i) Mammals other than primates

Phoenix et al (1959) examined the sexual behaviour of female guinea pigs whose mothers received testosterone propionate during pregnancy. When mature, the androgenized animals were castrated and given either progesterone and oestrogen or testosterone propionate (TP). With the former hormones androgenized females showed fewer lordosis responses than normal females. Without hormonal stimulation, only the androgenized females and castrated males showed mounting behaviour. With oestrogen and progesterone, normal females, males and androgenized females all showed mounting, but normal females took longer to show mounting than the others. When the androgenized females were given TP they exhibited mounting behaviour approaching male levels. These authors concluded that adult androgenized females were less likely to display female sexual responses and were more likely to display male

sexual responses, than were control females when both were administered the appropriate hormones. Although not stated by the authors, these findings furthered a widespread belief that masculinity and femininity were at opposite ends of a continuum. In other words, the guinea pig that shows a high probability and frequency of mounting behaviour and a low probability and frequency of lordosis (the behavioural characteristic of the receptive female) is considered masculine, and the guinea pig that shows lordosis, but not mounting is considered feminine. The androgenized female guinea pig shows increased mounting and decreased lordosis behaviour and is therefore considered both masculinized and defeminized. Goy, Bridson and Young (1964) extended this work, varying the dosage of TP given and the time of gestation at which it was administered. They contributed further to the belief that masculinization and defeminization were mutually inclusive when they showed that the greatest inhibition of female sexual behaviour occurred at the same time of gestation as the greatest enhancement of male sexual behaviour (Day 30). Treatments beginning on days 15, 20, 25 or 40 of pregnancy had lesser effects on the female guinea pig offspring. These results also suggested that prenatal TP treatment masculinized behaviour at the same time in development when it produced anovulatory sterility. Unfortunately, the latter suggestion was confounded by the interaction of dose and time of pregnancy and was not later confirmed by Brown-Grant and Sherwood (1971).

Work on androgenized female rats also added further weight to the masculinization/defeminization model (Harris and Levine, 1965; Gerall and Ward, 1966). But, in 1967, Whalen and Edwards showed that ovariectomized female rats given TP on the day of birth did not show

enhanced male behaviour in adulthood over controls (when both were given TP), while their lordosis response to oestrogen and progesterone was reduced. Thus, in rats, the presence or absence of post-natal androgenization stimulation failed to masculinize, but did defeminize and suggested that masculinity and femininity need not be opposite ends of the same dimension but could be independent dimensions of an animal's sexuality. In subsequent work on the hamster, "masculine" and "feminine" dimensions were similarly identified. The sexual differentiation of behaviour in the hamster is different to rats and guinea pigs, since in the latter two species if the adult male is castrated and given ovarian hormones, he is unlikely to show female behaviour, and if the adult female is ovariectomized and treated with androgens she is very likely to display male mounting responses. The opposite is true of the hamster. The adult female rarely shows mounting responses, even if given large doses of testosterone (Tiefer, 1971), whereas the male will show lordosis if given ovarian hormones (Swanson and Crossley, 1971), although usually of shorter duration than females. Thus the female hamster appears to be demasculinized during development but the male develops the potential to show both masculine and feminine responses. In this respect it was not surprising that the neural mechanisms controlling masculine behaviour in the hamster were found to be more sensitive to neonatal testosterone than those controlling lordosis behaviour (DeBold and Whalen, 1975). Several authors consider hamsters more useful than rats or guinea pigs as models for examining behavioural differentiation because normal females rarely show male behaviour (Swanson and Crossley, 1971).

Beach, Kuehn, Sprague and Anisko (1972) and Beach (1975) proposed that the masculinization and defeminization of behaviour were

two distinctly separate processes and that sexually dimorphic behavioural traits need not be mutually exclusive. Beach and Kuehn (1970) and Beach et al (1972) used either combined or separated pre- and post-natal androgenization treatments in dogs. All the animals were ovarhysterectomized and treated with oestrogen and progesterone as adults. They found that males castrated when adult and given testosterone mounted females, but were not mounted by males when given oestrogens, and ovariectomized females were mounted by males, but never mounted males when given testosterone. Under the same hormonal regime in adulthood, females treated with testosterone propionate during infancy were almost completely defeminized, but showed little increase in masculine behaviour. In contrast, females treated pre-natally showed a much greater enhancement of masculine behaviour and not as great a reduction in feminine behaviour. Clarke (1977) came to the same conclusion when he found that prenatal testosterone, (given to different groups of ewes at different times during gestation), was able to enhance masculine behaviour long after the stage of gestation at which it ceased to suppress oestrous. These androgenized ewes clearly showed male behaviour in the presence or absence of female behaviour and vice-versa.

The dissociation of behavioural components was also supported by Payne (1976). Single injections of testosterone (as opposed to TP) failed to produce behavioural defeminization (Luttge and Whalen, 1970; Coniglio, Paup and Gorski, 1973; Payne, 1976), whereas testosterone implants (Whalen and Rezek, 1974) or TP (Payne, 1976) were successful. This phenomenon was probably due to the more rapid clearance of free testosterone (Payne, 1976). Payne was therefore able to show that treating neonatal female hamsters with testosterone produced masculinized

individuals, whereas TP treatment produced both defeminized and masculinized individuals, emphasising that masculinization and defeminization were not mutually inclusive or exclusive. Whalen and Etgen (1978) came to similar conclusions and showed that dose-response analyses of the effects of hormone treatments were critical in order to reach these conclusions correctly.

From their experiments with sheep Clarke and Scaramuzzi (1978) took this one step further and proposed that prenatal androgenization produced a qualitative shift in behavioural potential which involved a loss of the potential to display feminine sexual behaviour and an aquisition of the potential to display masculine sexual behaviour. They found that injecting either testosterone or oestrogen into normal ewes produced only feminine behaviour (a 'female' brain response), whereas the same treatment with androgenized ewes produced masculine behaviour (a 'male' brain response). The 'brain sex' of the ewes also determined the degree of their aggressive behaviour, irrespective of the type of hormone they were given. The masculinized ewes showed more aggressive behaviour than the normal ewes, conforming to the fact that, as in most species, males are more aggressive than females (Bronson and Desjardins, 1971; Moyer, 1974; but see Payne and Swanson, 1970).

However, the effects of androgenization on sexual behaviour were questioned by Pfaff and Zigmond (1971). They pointed out that masculine behaviour had always been tested during testosterone treatment in adulthood, and no-one had shown whether pre- or post-natal androgen affected adult behaviours by modifying a "masculine behaviour-producing mechanism" or a "testosterone-receiving mechanism". Consequently they tested male, female and androgenized female rats for both feminine

and masculine behaviour after castration and injection with testosterone propionate, with oestradiol, testosterone propionate and progesterone, or oestradiol and progesterone. As predicted from previous studies (see above), androgenized female rats showed reduced feminine responses but failed to show enhanced masculine behaviour with either TP or oestrogen in adulthood. In contrast, other authors have clearly shown that androgenized female rats (Sodersten, 1973), hamsters (Johnson, 1975), guinea pigs (Phoenix et al, 1959), mice (Manning and McGill, 1974), dogs (Beach et al, 1972) and sheep (Short, 1974 a; Clarke, 1976) do show more masculine behaviour in adulthood than normal females. Furthermore, with ovariectomized female rats, Sodersten (1973) found that oestradiol benzoate or TP would stimulate mounting to a greater extent in androgenized females than in controls. The results are therefore contradictory. In an attempt to clarify the situation, the following experimental method exemplifies the problem of interpreting behavioural responses to hormones. Whalen, Edwards, Luttge and Robertson (1969) androgenized female rats and found that while androgenized females intromitted more than controls, their mounting frequency was not enhanced. However, the ovariectomized control females were given daily TP injections for up to seven weeks. In this case it was hardly surprising that they displayed high levels of mounting behaviour and obscured a possible masculine response from androgenized females. Prolonged testosterone treatment of normal adult female sheep will result in enhanced displays of male behaviour never observed in untreated ewes (Johnson, Hudson, Bogart, Oliver and McKenzie, 1956), and this is also true of the hamster (Noble, 1974). Nevertheless, in the end, Pfaff and Zigmond (1971) and Sodersten (1973) did agree that the effects of androgenization on adult females did alter specific behaviour rather than just the sensitivity to any particular hormone.

Clemens, Hiroi and Gorski (1969) and Brown-Grant (1975) emphasised the problems of interpreting behavioural responses when they found that a significant increase in receptivity ("willingness" to allow male mounts) of androgen-sterilized rats could be produced by manipulating test conditions. For example, Clemens et al (1969) allowed rats to adapt to the test cage for two hours before introducing a stud male. Their results showed no significant reduction in receptivity following neonatal androgenization, and seemingly place a limit to the measure of behavioural defeminization achieved. Beach (in press) reviews similar findings and stresses the need to evaluate exactly what each behavioural test shows and the fallacies of extrapolating these results too far, especially from one species to another (Beach, 1976).

Neonatal injections of oestrogen affect the behaviour of female rats in a similar manner to neonatal testosterone, and sometimes to an even greater extent (Harris and Levine, 1965; Whalen and Edwards, 1967; Mullins and Levine, 1968; Doughty et al, 1975a, 1975b). Increased mounting behaviour in female rats receiving neonatal oestrogen has also been reported by Hendricks (1969) and Hendricks and Gerall (1970), and by Paup, Coniglio and Clemens (1972) in female hamsters. These results support the theory that 'androgenization' of the female brain is mediated via aromatization of androgen to oestrogen (as previously discussed in Section 1.2b).

In a study by Whalen and Rezek (1974), testosterone implants were found to be more capable of inhibiting lordosis quotients of female rats than those of androstenedione. If 'androgenization' of sexual behaviour is mediated by the conversion of androgen to oestrogen,

then these results imply that testosterone is more effectively aromatized by the brain than androstenedione. In contrast, Weisz and Gibb (1974a) found exactly the opposite. However, this was from brain tissue taken from adult female rats. Data for neonatal females are not available, but if the aromatizing capacity of the neonatal brain is similar to the adult, the results of Whalen and Rezek (1974) would imply that testosterone per se exerts a specific androgenic effect on the brain tissues during development. Although the ability of neonatal female rat brain tissue to aromatize androstenedione and testosterone has been demonstrated (Reddy et al, 1974; Weisz and Gibb, 1974b), the conversion rates have not been compared in one system. Evidence for the theory that oestrogen is the active component in 'androgenizing' behavioural centres of the brain has been reinforced by the failure of a non-aromatizable androgen, dihydrotestosterone, to reduce the female sexual behaviour of neonatally treated rats (Korenbrot et al, 1975). However, this is not so clearly the case in other species. In hamsters, neonatally-administered dihydrotestosterone and androsterone (also non-aromatizable) decrease the duration of lordosis elicited by oestrogen, but are still much less effective in doing so than testosterone (Gerall, McMurray and Farell, 1975; Payne, 1976). Whalen and Etgen (1978) have also shown that aromatization may be necessary for defeminization. Moreover, unlike testosterone, dihydrotestosterone and androsterone do not increase aggression or male sexual behaviour when given neonatally (Paup et al, 1972; Payne, 1976). On the other hand, in guinea pigs, aromatizable androgens and not dihydrotestosterone are able to suppress lordosis behaviour, but the latter and other non-aromatizable androgens may have a role in

the development of masculine copulatory behaviour (Goldfoot and Van der Werff Ten Bosch, 1975). However, Whalen and Etgen (1978) have shown that at least in the hamster one should not immediately suppose that androgens stimulate masculinization and oestrogens stimulate defeminization. As previously suggested by Goy and Goldfoot (1975) male and female sexual behaviour appear subject to independent hormonal determination during development.

1.2c(ii) Primates (including man)

While our knowledge of behavioural differentiation in primates and Man is not as extensive as in rodents, the subject has been well-reviewed recently (Money and Ehrhardt, 1972; Money and Musaph, 1977; Diamond, 1965, 1968; Ciba Symposium No. 62, in press). Only the essence of this literature will be reviewed here.

Behavioural masculinization has been observed in genetic female rhesus monkeys (Goy and Resko, 1972), and girls (Money and Ehrhardt, 1972) exposed to elevated levels of androgen during fetal life. In rhesus monkeys, androgenization was achieved by administering androgens to the mother during pregnancy (Goy, 1968; Goy and Resko, 1972; Goy et al, 1977; Goy, 1978). In Man, workers capitalised on "experiments of nature" and androgenization was accidently achieved in several ways: (a) progestin administration (synthetic progesterone compounds, related in chemical structure to androgens, were given to mothers to prevent a miscarriage. When they were first synthetised, it was not known that certain of them could exert a masculinizing influence on a female fetus (Whalen, Peck and Lo Piccolo, 1966). Progestins (17- α -ethinyltestosterone and Norlutin: 17 α -ethinyl-19-testosterone) are similar to the highly virilizing compound of 17-methyltestosterone because they all have a 17-OH group in the beta position (Wilkins, 1960)):

(b) the virilizing adreno-genital syndrome (Because of a genetic defect the adrenals are, to a greater or lesser degree, unable to synthetise cortisol. This results in little or no cortisol negative feedback to the hypothalamic-pituitary unit, hence more adrenocorticotrophic hormone (ACTH) is released from the pituitary stimulating the production of cortisol precursor compounds, including adrenal androgens): or

(c) true hermaphroditism (where an individual possesses testicular and ovarian tissue).

With androgenized female rhesus monkeys, their pre-pubertal social and sexual behaviour and their post-pubertal sexual behaviour were affected (Goy and Resko, 1972). Pre-pubertally, androgenized females displayed typically masculine behaviour by exhibiting more active play behaviour (play initiation, the open-mouthed play face, pursuit or chasing play, and rough-and-tumble play), aggression (threat and dominance) and sexual behaviour (mounting, thrusting and erection of the penis) than did controls (Goy, 1968; Phoenix, Goy and Resko, 1968; Goy and Resko, 1972; Phoenix, 1974; Goy et al, 1977; Goy, 1978). Post-pubertally androgenized females showed typically masculine sexual behaviour (e.g. double foot clasp mounts), even to the point of masturbating and ejaculating fluid through the penis (Eaton, Goy and Phoenix, 1973; Phoenix, 1974). The latter behaviour was found when androgenized females were ovariectomized, given testosterone, and then presented with sexually receptive females. The mounting behaviour was also observed with androgenized animals in peer groups with or without their mothers present (Goy, 1978). Unfortunately, there is no information as to whether the feminine behaviour of androgenized females was suppressed.

When rhesus females were exposed to testosterone postnatally (6.5 - 14.5 months old; n = 3), play and mounting behaviours were unaffected (Joslyn, 1973). Nevertheless, the injected females gained in dominance status among their peers and during that change showed increased aggressive behaviour. They maintained their elevated status long after the testosterone injections stopped. It is unclear whether the increased social dominance in these infant female rhesus monkeys was caused by the effects of testosterone on the central nervous system, peripheral structures (e.g. increased body size and enlarged clitoris), or both, or whether by the end of the hormone treatment, this behaviour was so well learned that it became independent of hormone support. Unfortunately, this study did not examine the early postnatal period, from about 1-14 weeks of age, when male rhesus monkeys experience a high level of circulating testosterone (Robinson and Bridson, 1978).

But, the prenatal (and perhaps postnatal) hormone environment is not the only factor that affects dimorphic behaviour in rhesus monkeys. Goldfoot and Wallen (1978) have shown that rearing conditions and rank in the dominance hierarchy have a large effect on the degree to which animals display sexual, playful and aggressive behaviour. They found that the presence of peers of the opposite sex influenced the development of foot clasp mounting. Females reared away from males (in iso-sexual groups) were more likely to display foot clasp mounting, whereas males reared away from female peers were less likely to display the response. Normally, in heterosexual groups, males display foot clasp mounting much more frequently than females (Goldfoot and Wallen, 1978). Furthermore, males in heterosexual groups engaged in three times the number of rough play bouts than females in the same groups

or iso-sexually raised males. Iso-sexually reared females display even less rough play than the latter. In heterosexual groups, males consistently occupy the higher dominance ranks and threaten and attack their peers more frequently than males or females in any other condition of rearing. In contrast to males, high-ranking females (in iso-sexual groups) rarely attacked their peers. These results illustrated that a proportion of sexually dimorphic data from mixed sex groups of rhesus monkeys could be explained by dominance rather than sex factors. For some categories of response the behaviours were dimorphically reversed when low ranking males were compared with high ranking females. Secondly, certain dimorphic behaviours observed in one situation were not always dimorphic in another, emphasising that assessments of behavioural dimorphism should be related to the exact rearing and testing conditions, and that the postnatal social environment might also serve in determining the eventual gender profile of rhesus monkeys.

In the human, the postnatal environment plays an even greater part in determining an individual's ultimate gender identity. Fetally androgenized girls reared as females showed masculinized prepubertal behaviour when compared with matched controls (Money and Ehrhardt, 1968, 1972; Ehrhardt and Baker, 1974). A significant number were "tomboys" (i.e. they displayed high levels of physical energy, especially in vigorous outdoor play, games and sports (commonly considered the perogative of boys), preferred male to female playmates and trousers to dresses). They also significantly preferred toy cars, guns etc. to dolls, and subordinated marriage to career prospects. Girls androgenized by the adreno-genital syndrome also showed a lack of satisfaction with their female role, had very little interest in taking

care of infants, had fewer daydreams or fantasies of pregnancy and motherhood, and wedding and marriage were not anticipated in their play and daydreams. But fetal androgenization did not, per se bring about a change in the childhood or adolescent sexual play of these girls. (Money and Ehrhardt, 1972). Recently information has become available comparing girls born to mothers given primarily progestins (androgenic compounds) during pregnancy and those born to mothers given mainly oestrogen (Reinisch, 1977). By about 12 years old, girls exposed to progestins were significantly more independent, sensitive, individualistic, self-assured and self-sufficient than those in the oestrogen group. However, in contrast to an earlier report (Money and Ehrhardt, 1968) there was no significant difference in IQ.

In adolescence, fetally androgenized girls tended to lag behind their peer group in developing sexual relationships with boys, and did not have the precursor interest in romance and boyfriends from their play and dreams to carry forward into dating. But, in contrast to the rhesus monkey and non-primate species (see above), their sexual behaviour was not masculinized. The same was true of women chronically virilized externally by the adreno-genital syndrome until adolescence or later into adult life (corrective cortisone therapy which reduces adrenal androgen production was not started until 1950). While 10 out of the 23 latter individuals had homosexual as well as heterosexual dreams and fantasies (a relatively high incidence according to Money and Ehrhardt, 1972), and four reported bisexual experiences, none of them considered their female gender identity to be incorrect and none had entertained the idea of a sex change (Ehrhardt, Evers and Money, 1968). Thirteen of the 23 married and five became pregnant. Their babies were normal and at least one mother breastfed (Money and Raiti,

1967). If androgenized girls were reared as males, they adopted a masculine identity (Money and Ehrhardt, 1972).

Such examples emphasise the importance of rearing in dealing with studies of human behaviour and contrast with those of lower mammals because fetal androgenization of human females does not automatically reverse gender dimorphic behaviour. Apparently the brain centres for behaviour in Man are either less sensitive to these effects, or prenatal influences can be over-ridden by postnatal experience. This is further complicated as these individuals usually have their internal and external genitalia altered to suit their assigned sex at birth, with or without the appropriate hormone replacement therapy (Money and Ehrhardt, 1972). Thus, in the human, it is very difficult to study behavioural effects of the prenatal environment without at least several postnatal factors confounding the results. Three matched pairs of androgenized girls, concordant for diagnosis (adreno-genital syndrome), but discordant for sex of rearing have neatly demonstrated the heavily weighted contribution of the postnatal phase of human gender differentiation (Money and Ehrhardt, 1972). In the first pair, one child was reared as a girl, given cortisone therapy and her external genitalia were surgically altered to a female appearance (including removal of the clitoris and opening of the vagina). This was accomplished by two years of age. The other was reared as a male, 'his' external genitalia were surgically transformed to resemble those of a male (including a penis and prothetic testes inserted into a 'scrotum') and 'he' was given androgen therapy at puberty. Both children have kept their given identities. In a second matched pair, one child was reared as a girl, but without corrective cortisone therapy (adrenal androgens were not

suppressed) and without surgical transformation of the genitalia. The other was reared as a male, again without surgical transformation of the genitalia, but with corrective cortisone therapy (suppressing 'his' adrenal androgens; 'he'was mistakenly thought to be a genetic male suffering from the adrenogenital syndrome). Both children were studied from 12 years old. At this age the child reared as a girl viewed the masculinization of her body occurring at puberty as a deformity and reacted against it. She was then given corrective cortisone therapy and her external genitalia were surgically feminized (the vagina was opened and the enlarged clitoris removed). She married fifteen years later. The child reared as a boy was disgusted with 'his' breast development at puberty ('his' adrenal androgens had been suppressed but 'his' developing ovaries ensured normal female development) and was enthusiastic to receive testosterone therapy and a mastectomy. Surgical alteration of his external genitalia was not necessary because his penis and scrotum were sufficiently developed. He had a girlfriend and experienced erotic arousal from being with her and watching other girls. In this pair, the sex of rearing again predominated.

The third matched pair illustrate the principle that indecisive sex assignment lays the foundation for ambivalence or discordance of gender identity with the ostensible sex of rearing (Money and Ehrhardt, 1972). In neither case were the parents given a clear assignment of sex to their babies; no baby underwent corrective surgery to its genitalia, and both received little or no cortisone therapy. The child reared as a male elected to become a female at 12 years old (both children were regularly interviewed by doctors at Johns Hopkins' Hospital, Baltimore, U.S.A.). This female, following corrective

therapy, is now happily married and plans to become pregnant. The child reared as a female, eagerly opted for reassignment to a male after coming to the attention of the authorities at 11 years old. At this age the child looked like a 14-15 year old boy (precocious puberty brought on by the excessive adrenal androgens). With 'his' new identity, this individual was attracted exclusively to girls and began dating at 15.

These three cases of genetic females, suffering from the adrenogenital syndrome, emphasise the prerequisite that, with humans, parents must have no doubt or ambiguity as to whether they are raising a son or daughter. This is because children with prenatal abnormalities who eventually request a reassignment, typically have a history of uncertainty as to their sex of assignment, and their requests are made irrespective of genetic and gonadal diagnosis (Money and Ehrhardt, 1972).

Nonetheless, girls unambiguously raised as female but androgenized during fetal life, do show significant masculinization of prepubertal behaviour, even though their gender identity is irrevocably feminine (Money and Ehrhardt, 1972). Androgenized female rhesus monkeys, on the other hand, are apparently masculinized in all behavioural respects (Goy, 1978). However, neither develops the anovulatory syndrome (Goy and Resko, 1972; Wilkins et al, 1955; Wilkins, 1960). Hence behavioural masculinization would appear to occur independently of hypothalamic sexual differentiation in at least these higher mammals.

In further contrast to lower mammals, genetically female rhesus monkeys were as effectively masculinized behaviourally by dihydrotestosterone (a non-aromatizable androgen) as by testosterone

(Goy et al, 1977; Goy, 1978). So apparently in the rhesus monkey, and maybe in the human, androgens can have a direct effect on the behavioural centres of the brain without first being converted into oestrogens. In this respect it is interesting to note that the prenatal social behaviour and postnatal sexual behaviour of testicular feminization patients (genetic males insensitive to testosterone) does not differ from that of normal women (Diamond, 1968; Masica, Money and Ehrhardt, 1971). However, their hypothalamus is masculinized, suggesting a possible oestrogenic effect (see Section 1.2b(ii) for discussion). The precise roles of androgens and oestrogens in the sexual differentiation of Man and primates still await clarification.

Hence, in general, sexual differentiation of the brain centres controlling behaviour is dependent upon the presence or absence of androgens (or their metabolites) during early development (except in Man where postnatal rearing can override a fetal history of androgenization). The process whereby masculine behaviour is enhanced in androgenized females is separate from that promoting behavioural defeminization. Furthermore, behavioural and anatomical androgenization of females may occur in primates, such as the rhesus monkey and Man, without impairment of the ovulatory control mechanism.

1.3 The aims and scope of the study

In primates, experimental investigations of sexual differentiation have been mainly confined to the rhesus monkey (Goy, 1968; Phoenix et al, 1968; Goy and Resko, 1972; Goy et al, 1977; Goy, 1978). This monkey is a highly polygamous species with marked anatomical and behavioural sexual dimorphism. The common marmoset, on the other hand, is an example of a monogamous monkey which exhibits little

anatomical or behavioural dimorphism. Since its monogamous social structure may resemble the human norm of serial monogamy (Short, 1976a; Kleiman, 1977) more closely than does the extreme polygamy of the rhesus monkey, it is of particular interest to investigate sexual development, especially sexual differentiation, in this primate.

This study was therefore undertaken to establish criteria for the normal physical, hormonal and behavioural development of common marmosets from birth to maturity and ultimately to examine the effects of testosterone on sexual differentiation. Because of the lack of sexual dimorphism in this monkey, and the fact that females neither menstruate (Hearn and Renfree, 1975) nor show any obvious signs of oestrus (but are spontaneous ovulators with a cycle length of 16.4 $\pm$ 1.7 days; Hearn and Lunn, 1975), detailed measurements of growth, sex hormones, and social and sexual behaviour were required in order to achieve a comprehensive understanding of marmoset sexual development. Secondly, as only the dominant male and female breed in each group (Epple, 1967; Rothe, 1975; Abbott and Hearn, 1978), the effects of social dominance on sexual development and mature reproductive behaviour had to be explored to understand how these latter two categories were affected by this monkey's monogamous social system. Finally, having established criteria for normal development and the effects of dominance, the effects of testosterone on sexual differentiation were considered. Now, both intact adult male and female marmosets exhibit a positive feedback response of LH to oestradiol administration (Hodges and Hearn, 1978). So this response is not sexually dimorphic. These monkeys also have a gestation period of 144 $\pm$ 2 days (Chambers and Hearn, in press) and usually give birth to dizygotic twins

(Hearn, Abbott, Chambers, Hodges and Lunn, 1978). The twin embryos establish vascular anastomoses between their placental circulations within a few days of implantation (Wislocki, 1939), and hence they develop as haematopoietic chimeras (Benirschke and Brownhill, 1962; Benirschke, Anderson and Brownhill, 1962; Gengozian, Batson and Eide, 1964). Nevertheless, females born chimeric to their male co-twins are not adversely affected as a result of this: the freemartin condition does not occur (Wislocki, 1939; Benirschke and Layton, 1969), their ovaries and genital tract are normal, and there is no evidence of hypertrophy of any Wolffian duct derivatives (Wislocki, 1939). These females also copulate and breed normally (see Chapter 6).

In other primates, such as the rhesus monkey and the human (as mentioned earlier), normal male fetuses experience increased concentrations of plasma testosterone during early to mid-gestation, when sexual differentiation might be occurring. Male rhesus and pig-tailed macaques and human males (see above) also show a brief elevation of testosterone levels neonatally. As yet there is little information on blood testosterone in early male marmoset fetuses, but in fetuses of 80-90 days gestation and over, there is no difference in testosterone between males and females (P. L. Chambers, personal communication). The gonads differentiate at about 70 days of gestation (W-S. O, P.L. Chambers and J.P. Hearn, personal communication). Moreover, the newborn male experiences raised levels of plasma testosterone soon after birth (Abbott and Hearn, 1978), and the female does not. It is therefore possible that this monkey has delayed the sexual differentiation of its brain until after birth. In order to

discover whether neonatal androgen treatment could influence sexual differentiation, newborn female marmosets were implanted with testosterone and the masculine and feminine character of these offspring were studied.

The same monkeys were used in many parts of the study so that an accumulation of behavioural and endocrinological data could be correlated within the same individuals.

CHAPTER 2 MATERIALS AND METHODS

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2.1 Animals

The common marmoset monkeys used in this study (Callithrix jacchus jacchus, as classified by Napier 1976) were maintained at the MRC Unit of Reproductive Biology Primate laboratory at the Bush Estate, Midlothian. The adults were over 2 years old and were either caught in the wild more than $2\frac{1}{2}$ years before their use in observations, and fully adapted to captive conditions, or born in captivity. All the young animals were born in the colony.

2.2 Management

Marmosets were kept in aluminium cages containing a nest box and wooden perches. In each of the four rooms, animals were allowed weekly access to a large exercise cage (2.0 x 1.0 x 2.0m) filled with branches, runs and swings. The exercise cage was connected to the home cage by flexible ducting, for 12-24h at a time. Each room was maintained at a temperature of 24°C (range 21-29°C) and 65% relative humidity (range 55% - 70%), and the ventilation system changed the air about 10 times an hour. The animals were kept in natural light supplemented with artificial illumination between 07:00h and 19:00h . Hearn, Lunn, Burden and Pilcher (1975) have already reported the full details of management in this colony, including aspects of diet, vitamin supplements and maintainance routine.

2.2a Families

Family groups were kept in double cage units (100 x 50 x 75cm; Hearn et al,1975), and were usually limited to the parents and four offspring. Adult females usually produced offspring twice a year and 60 - 80% of these were twins (Hearn et al, 1975; Lunn and Hearn, 1978). Hence, in order to prevent overcrowding and to establish new breeding groups, the offspring were removed from their families

between 300 and 500 days of age. They were either housed in peer groups or paired with an animal of the opposite sex. Conveniently, young animals reached puberty at about 450 days of age (Kingston, 1975).

2.2b Peer groups and pairs

Peer groups usually comprised 3 male and 3 female marmosets born in different families in the colony. They were also housed in double cage units. This number was normally reduced to five after one or two days, when one of the group was usually removed because of attacks from other group member(s). After the birth of offspring in the group, the parental pair were left with their young to form the nucleus of a new family group, and the remaining males and females were either paired together or with a sexually experienced adult of the opposite sex.

Marmosets in male-female pairs were kept in single cage units (50 x 50 x 75 cm; Hearn et al, 1975) and if the females did not become pregnant or did not rear young within 8-12 months, their partner was changed.

2.3 Observation room

2.3a Design

The room was designed to allow easy observation of these arboreal monkeys through a one-way mirror, in an area fitted with branches and perches (Figure 2.1). The walls of the observation partition were angled so as to maximise the area of vision from the observer's window. All the partitions were pre-made in sections to permit easy assembly and dis-assembly.

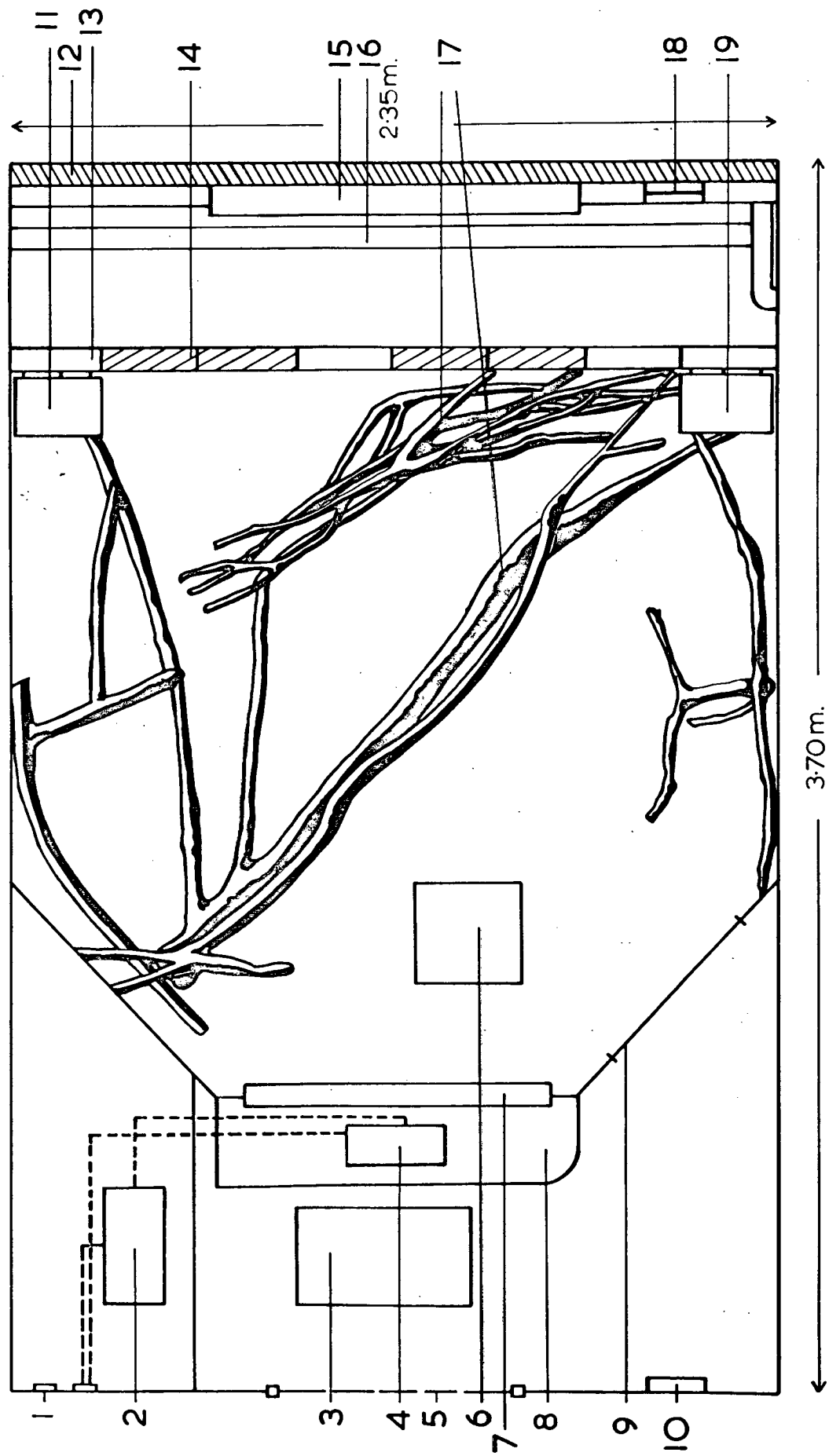
FIGURE 2.1: The observation room.

(a) Plan view.

KEY

1. Mains power supply (13 amp)
2. Tape recorder
3. Observer
4. Keyboard
5. Door (into corridor)
6. Wooden stool
7. Observation window
8. Shelf
9. Door (into observation area)
10. Air input vent
11. Nest box (housing animals)
12. Exterior window
13. Window wall partition
14. Partition doors
15. Radiator
16. Heating ducts
17. Tree branches in observational area
18. Air vent (outflow only)
19. Nest box (housing animals)

FIGURE 2.1a



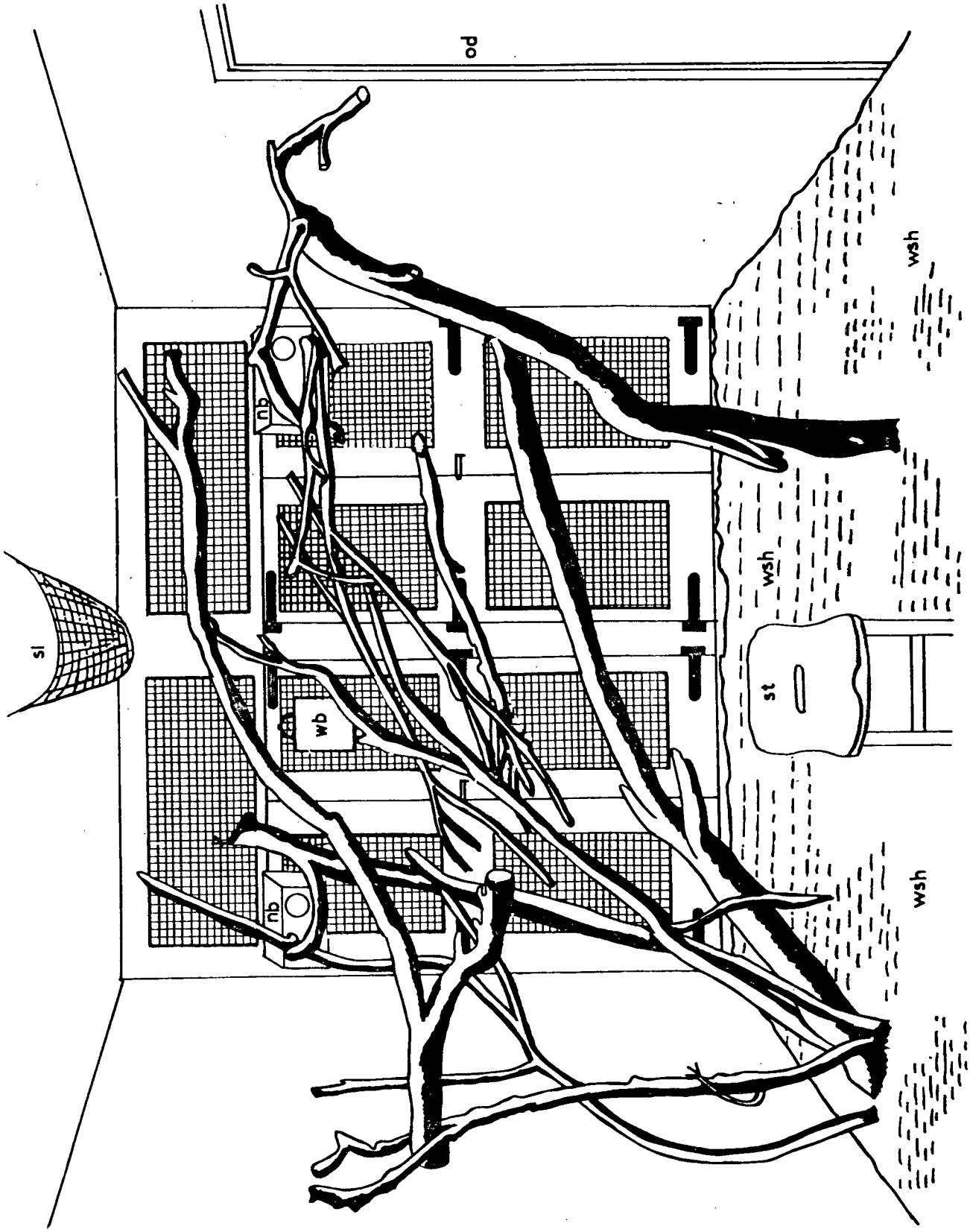


FIGURE 2.1: The observation room.

(b) View through the observation room.

KEY

nb	=	nest box	st	=	wooden stool
wb	=	water bottle	sl	=	strip light
wsh	=	wood shavings	od	=	access door to the observational area.

Original drawing by courtesy of Frank J. Burden.

2.3b Construction

The wooden panel at the observor's end of the room was fitted with a 0.9m x 1.2m sheet of glass (0.3cm thick). The glass was coated with silver tinted Madico film (City Glass Co. Ltd., Glasgow) to reduce the transmission of visible light by 83%. As the only sources of light were on the animals' side of the partition (a large strip-light and three windows covered with translucent perspex), the coated glass effectively became a mirror to the animals and a window on the observer's side. However, sufficient light penetrated the glass-film to permit the observer to operate the recording equipment or make notes (see sections 2.4, 2.7 and 2.8).

The strip-light and heating and ventilation ducts were also partitioned off from the monkeys (Figure 2.1). The room was then equipped with an assortment of branches while the floor was covered with wood shavings.

2.3c Routine use

When marmosets were introduced into the observation room, their own nest box and water bottle were hung on the window wall partition but no food was provided. A wooden stool (60cm high) was placed in the middle of the room to allow the animals to use this area without using the floor as a means of access (Figure 2.1). After each visit to the room, the animals were herded back into their nest box (with the aid of a net) and taken back to their home cages. Two months before observation began, the room was regularly used as an 'exercise cage' in an attempt to habituate the animals to the fresh branches. Only occasional wiping of the walls and branches was necessary to keep the room clean and the shavings on the floor were changed every 3 months. The room was never washed out.

2.3d Behavioural test conditions

The conditions were identical to those for routine use except no water was provided for the short periods (15 min. per test).

2.4 Behavioural observation of families

2.4a Observation room

Family nest boxes were suspended by hooks to the window wall partition. The animals were released into the room by the observor, who retired behind the observational panel within 5 seconds.

2.4b The observation schedules

Families were observed when one of their sets of twins (or triplets) offspring were 40-50, 80-100, 150-180, 250-280, or 350-400 days of age. The youngsters concerned were at least the second set to have been reared by their parents. Usually four observations were made at every age. An initial 30 min. observation (made in the "morning" or "midday") preceded the rest, which were each allocated to one of the three periods of the day ("morning" 09:30-12:00h, "midday" 12:00-14:30h, and "afternoon" 14:30-18:00h). Each observation was carried out on a different day and the observation times were chosen to avoid disturbance of the colony maintainance routine, and to minimise diurnal variations in behaviour. Normally the animals were active between 05:30-07:00h until 18:00-19:00h, but usually showed a period of inactivity of 0.5-2.0h between 11:30h and 15:00h (similar to reports by Stevenson and Poole, 1976).

During the initial observation of 30 min., general notes were made on all the members of the family (see 'Behaviour recorded' in section 2.4d). This period of time aclimatised the animals to the observation room. The aggressive threats and submissive gestures,

mainly displayed by the parents at their reflections in the observation window, dropped to a constant low level in this time (Figure 2.2; see 'Behaviour recorded' in section 2.4d). In each of the remaining three observations general notes on all family members were also made during the first 30 min. while displays at the window again dropped to a minimum. Then for the succeeding 30 min., one of the twins under study was observed as the 'target animal'. Only its behaviour and the animals it interacted with were scored to the exclusion of the rest of the family. Exempt from this ban were events such as mating, play and dominant-subordinate interactions. Furthermore, if the 'target' animal was seldom active, the behaviour of its twin and other family members was also recorded. During the following 30 min., the other twin became the 'target' for observation. If triplets were observed, a further 30 min. of observation was necessary, and if for any reason only one member of a set of offspring was left alive, it was randomly designated as the 'target' during the second or third 30 min. period of each observation.

The order in which the 'target' animals were observed was randomised by tossing a coin. A series of 500 outcomes (i.e. 'heads' or 'tails') was achieved on a double-blind schedule; the ratio of heads:tails was as expected by chance (χ^2 test; df = 1; $p < 0.6$). A twin in each family was designated either 'heads' or 'tails' and the order of the outcomes in the 500-series determined which was observed first on every occasion. When each head or tail outcome was used it was scored off. For example, if 'heads' was the next outcome on the list, then the twin so designated was observed first. This procedure was repeated for the next observation period at the same age, but if the same twin had been watched first in both of the second and third

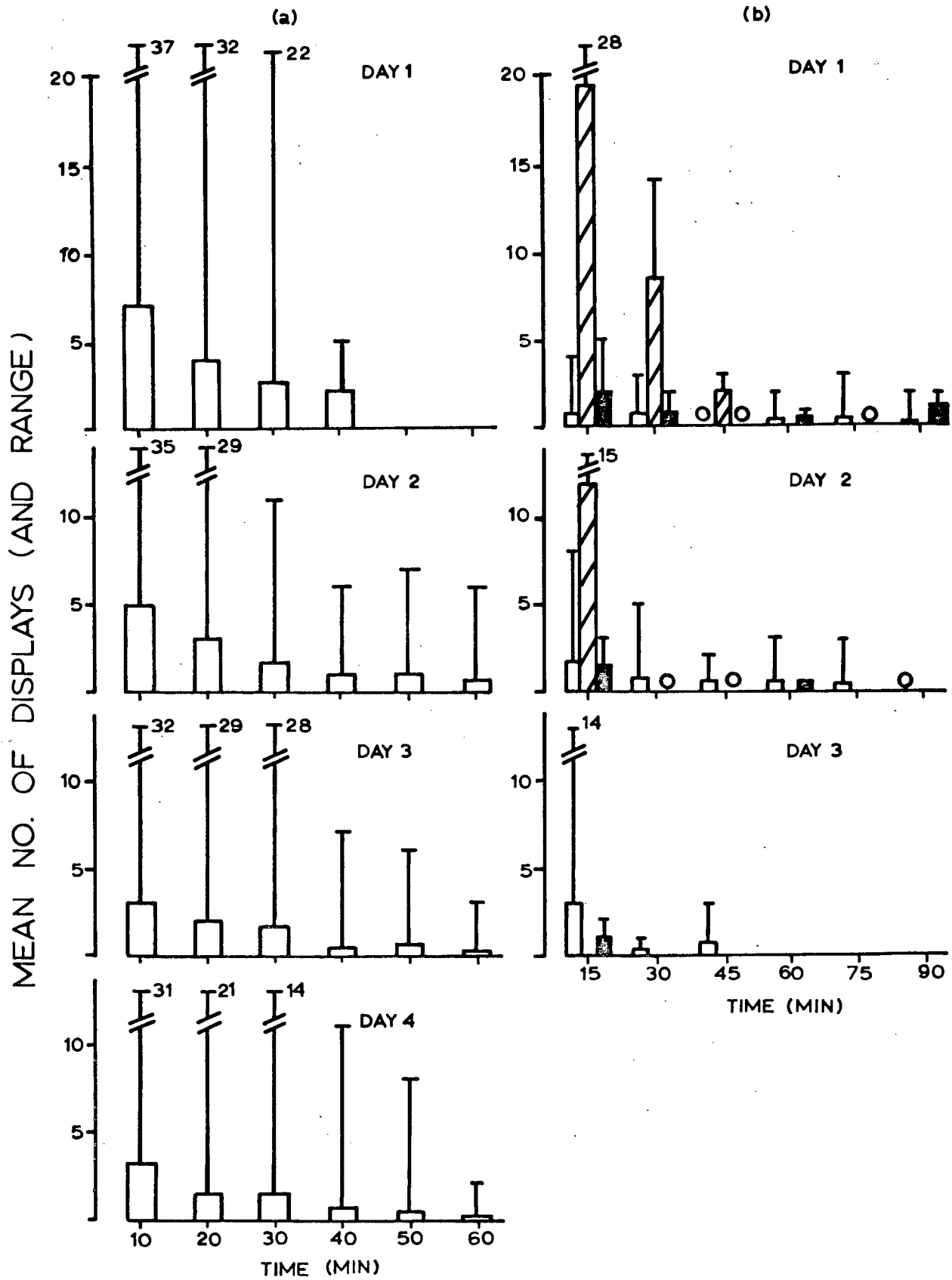


FIGURE 2.2: Aggressive displays (a) by families at the observation window and (b) by peer groups at the observer or observation window.

In (b) ▭ = displays at observer when group first established (see text)

▨ = displays at observer 3 months after group established

▩ = displays at observation window 3 months after group established.

periods, then the other twin was automatically watched first in the last observation, regardless of the next head or tail outcome on the list (this outcome was scored off anyway). If only a single animal was left in the set of offspring under study, then the order of heads and tails designated whether or not it was observed in the second or third 30 min. period of each observation, with an analogous option in the last observation as for the twins. If the triplets were under investigation, a die was thrown to decide the order of observation, with one of the twins designated 1 & 4, the second 2 & 5, and the third 3 & 6. This was done for every observation (only one set of triplets was involved; see Chapter 6).

2.4c Identification of animals

Physical size readily distinguished the adults and each set of offspring. Hence only the tails of one parent and one of each set of offspring were shaved for identification. About 1.5-2.0cm of the pelage in the middle of the tail was removed with an electrical razor. With triplet offspring, one of the youngsters was given two such marks.

All the animals in the colony over 200 days of age carried coloured number tags on a chain around their necks, but these were not obvious from a distance.

2.4d Behaviour recorded

Recorded behaviour was divided into four sections - playful, sexual, aggressive and submissive. Similar marmoset behaviour has been compiled into an ethogram by Stevenson and Poole (1976) and their equivalent behavioural terms will be given when appropriate.

2.4d(i) Social play behaviour

This was a vigorous activity involving non-aggressive interactions with a partner or partners. At times the whole family would join in. When animals engaged in social play they usually flattened their ear tufts against the side of the head and half-closed their eyes from time to time, especially in rough-and-tumble play and hide-and-seek. They would also cavort around with their mouths gaping open, especially in rough-and-tumble and chasing play. As suggested by Stevenson and Poole (1976), this open-mouthed play-face of the marmoset may be similar to that of many other mammalian species (van Hoof, 1967; Jolly, 1972; Bekoff, 1972; Redican, 1975). In the open-mouthed expression, the teeth were visible, but the lips were not retracted, quite unlike the facial grimace associated with submissive behaviour where the lips were retracted ('bared teeth gecker face' of Stevenson and Poole, 1976). For the purposes of this study, marmoset play was divided into three categories as follows:

(a) rough-and-tumble : contact play involving wrestling (grappling and struggling with other animals combined with inhibited biting) and sparring (hitting of the upper parts of playmates' bodies with the arms or hands);

(b) chase : where an animal scampered closely or ran after another (or others), and

(c) hide-and-seek : where one animal (or animals) hid from another (or others) while being actively pursued. The hidden animal(s) would frequently expose its (their) head(s) to see its (their) pursuer(s) before ducking back under cover.

Between events of rough-and-tumble play, animals sometimes threw themselves backwards against say a branch or wall (sometimes almost horizontally), with their arms and sometimes legs outstretched

(prone). This was accompanied by the 'play-face' and was usually followed by animals rough-and-tumbling with the prone animal or the latter initiating rough-and-tumble play with members of the family (as found by Stevenson and Poole, 1976). Prone was not included in the category of rough-and-tumble because contact play was not involved.

The animal initiating each play category was defined as whichever exhibited the play behaviour to its play partner or partners first. In rough-and-tumble this was the animal which pounced on, wrestled with, grasped or sparred with a play-mate first. In chase it was the animal which first galloped rapidly away from its partner, but if the pursuing animal ran at this animal first before it galloped away, this was also noted. With hide-and-seek it was whichever of the animals that first went into hiding. Between every pair of playing animals, each category was considered terminated if interrupted by either of the other two types of play, or if one animal ceased playing with the other.

2.4d(ii) Sexual behaviour

Courtship behaviour: Before copulating, marmoset monkeys usually displayed some form of this behaviour:-

(a) pursuit courtship: where males closely followed females (within 30 cm.) displaying rapid and rhythmical protrusion of the tongue ('Zungeln'; Epple, 1967 : 'tongue in/out'; Stevenson and Poole, 1976) with lip-smacking behaviour ('Lippenschmatzen'; Epple, 1967 : 'lip-smacking'; Stevenson and Poole, 1976). Clearly audible and rhythmical sharp inhalations of air accompanied this behaviour, and frequently both animals displayed piloerection ('bristle'; Stevenson and Poole, 1976; when the body and tail pelage is erected).

(b) passive courtship: when an animal (either male or female) remained almost stationary and displayed rhythmical and rapid tonguing and lip-smacking movements, usually while nuzzling, licking and rubbing the partner's head, face, genitals and body.

(c) invitation to mount: where females used tonguing and lip-smacking behaviour as an invitation to the male to mount (similar behaviour has been described by Stevenson and Poole, 1976). In this situation females remained stationary at a distance from the males (more than 10 cm.; greater than arm's length), stared at their partner, flattened their ear tufts and half-closed their eyes, and displayed tonguing and lip-smacking behaviour (limbs and body motionless). Frequently females froze in a crouching position while displaying this behaviour (crouch), as if in a trance, and occasionally turned their heads sideways and back over their shoulders (head turning; commonly observed with females during copulation. However, the extent to which females adopted the crouch position was variable).

Copulation: Copulations were identified as 'mounts accompanied by pelvic thrusting' (mount and pelvic thrusting). Males always mounted the back of their female partners. Males initiated mounting by clasping the female around her shoulders, or just beneath her shoulders, with their hands, and then straddled the female's hindquarters with their nearest leg. Once mounted, males frequently moved their hands to grip the female around the waist. The male would also use his feet to clasp the female's legs or the surrounding substrate (quite different to the 'double foot clasp mount' of the male rhesus monkey; Goy and Resko, 1972). The male proceeded to exhibit rapid, rhythmical pelvic thrusting. At this stage, females often exhibited head turning,

and nuzzled and licked the male. It was difficult to observe if intromission took place. However, vaginal washings were examined for the presence of spermatozoa (see section 2.10). Copulations were terminated when either the male dismounted, or the female moved off, disengaging herself from the male.

Males might attempt to mount females (attempted mount) by putting their arms around the female's neck or shoulders while starting to straddle her hindquarters. At this point (see Chapter 6), the male might disengage from mounting the female or the female might reject his attempted mount, and not allow it to continue. Mounts or attempted mounts were considered rejected (reject mount) if the animal being mounted objected physically and/or vocally within 2-3 sec. of the male attempting to mount. Physical objections included pushing the male away, biting him, or just wriggling free. Vocal objections were voiced as aggressive threats or loud squeals (see 'Aggressive and Submissive' behaviour sections). A mount was considered accepted if there was no active resistance from the female within 2-3 sec. of the male attempting to mount. Female marmosets normally play a passive role during copulation.

2.4d(iii) Aggressive and submissive behaviour

Table 2.1 delineates the aggressive and submissive behaviour recorded.

2.5 Behavioural observation of peer groups

2.5a Observational cages

The peer groups were initially observed, without using a hide, in an exercise cage of 2.0 x 1.0 x 2.0m connected by flexible ducting to their home cage (see Figure 2.3 and section 2.2). Cage sizes were

Table 2.1: A description of the aggressive and submissive behavioural categories recorded in this study of marmoset monkeys.

BEHAVIOUR	DESCRIPTION	PREVIOUS DESCRIPTION
Aggressive:		
vocal threat	Low pitch sticcato chattering, commonly given preceding an attack on another animal.	"Chatters when angry"* "erh-erh"**
threaten and pursue	One animal closely pursues another while issuing vocal threats (usually accompanied by piloerection and erect ear tufts). Pursuit is different to follow because of the aggression ensuing the former.	-
ear tuft flicking	Flattening of the white ear tufts against the side of the head, followed by their sudden erection (the ear tufts are not always fully depressed before erection).	"Tufts-flick stare"**
genital present	The tail is raised and the genitals are exposed to another animal (usually accompanied by piloerection).	"Tail raised present"**
snap bite	A single sharp short bite normally delivered to the neck region of another animal. The aggressing animal might also grasp its victim.	"snap bite"**
cuff	A swift and superficial blow or scratch.	"cuff" **
attack	One animal pounces on another and bites, claws and wrestles violently with it, normally resulting in bruising of the animals and injuries which bleed.	
Submissive:		
facial grimace	The mouth is partially opened and the corners of the mouth are retracted, exposing the lower and sometimes the upper set of teeth.	"bared teeth gecker face"**
submissive vocalisations	Exaggerated forms of the infantile "ngä, ngä", "tsik-tsik" and squealing (these can accompany all submissive behaviours).	"ngä, ngä", "tsik-tsik", and "squeal"*
flattened ear tufts	Lowering of the ear tufts against the side of the head.	"tufts flatten"**
flee	Rapidly leaving the vicinity (approximately 30 cm.) of another animal	-

* Epple (1968)

** Stevenson and Poole (1976)

FIGURE 2.3: An exercise cage linked to one of the home cages.

identical in each of the 4 rooms used and the exercise cages remained connected to the home cage for 1 week after each group was set up. All peer groups under observation were in full view of the 40-60 marmosets in the same room, and each group had access to the exercise cage at least once a week after completion of the above schedule. The exercise cages were cleaned once a month in keeping with the maintainance routine.

The groups were observed again, three months after they were set up. This time they were installed in the observation room in a similar way to families (section 2.4a). This method was necessary because of the frequent aggressive threats directed by members of the group at the observer when observed in their exercise cages. This response was quite different to when the groups were first set up (Figure 2.2).

2.5b Observation schedule

Aggressive behaviour was rarely seen after the first 2-3 days. Hence groups were either observed for 2h after the animals were placed together in the exercise cage, for 2h on the next day, and for one hour on the third day, or they were observed for 1-2h. after the animals were placed together and just for ½-1h. on the next day. The latter schedule was brought into operation when it was found that it could yield relatively similar results to the original (see Chapter 5).

When groups were observed 3 months later, they were either observed for 2h. a day on 3 consecutive days or 1h./day on 2 consecutive days. Both schedules obtained relatively similar results.

All observations took place between 11:00h and 16:30h.

2.5c Identification of animals

Each animal was identified by the colour of its ear tufts. These were normally white, but were dyed with 1% Eosin Y (red) in the case of males and with 5% light green for females, as illustrated in Table 2.2. The dye was applied with a cotton-wool swab while the animal was manually restrained, and the ear tuft pattern was randomly assorted within each sex.

2.5d Behaviour recorded

Aggressive, submissive, social and sexual behaviour was recorded in the peer groups. The former two behaviours were used to determine the rank order in each group from dominant-subordinate interactions (see Chapter 5) and the latter two were used to identify the breeding pair.

Aggressive and submissive behaviour

These behaviours are outlined in Table 2.1.

Social behaviour

proximity: where an animal remained within arm's length of another ($\leq$ 10 cm.).

follow: when one animal followed another. The following animal kept its gaze fixed firmly on the one in front and no distance criterion was set. This behaviour was commonly associated with piloerection and the arched-back posture.

huddle: when animals rested next to each other, keeping bodily contact (Figure 2.4).

social grooming: where one animal groomed another, ("allogrooming"; Rothe, 1971). The groomer sifted through the fur of the groomee with its hands (sometimes combing the fur with its incisors). The groomer

Table 2.2 Identification of marmoset monkeys in a peer group.

COLOUR OF THE EAR TUFTS							
MALES				FEMALES			
ANIMAL	RIGHT EAR[+]	LEFT EAR[+]	ABBREVIATION[‡]	ANIMAL	RIGHT EAR	LEFT EAR	ABBREVIATION
A	RED	RED	RR	A	GREEN	GREEN	GG
B	RED	WHITE	RW	B	GREEN	WHITE	GW
C*	WHITE	RED	WR	C	WHITE	WHITE	WW

* If there were more than 3 animals of either sex in a group, one of the extra animals was coloured WHITE-GREEN (WG) and the remainder were dyed with a solution of Methylene Blue (BB, BW, WB).

[+] The animal's right or left as it was viewed face on.

‡ The symbol recorded in abbreviated notes.

FIGURE 2.4: An adult female and male marmoset huddling together.

also sporadically licked the groomee's skin and fur, and masticated, apparently on objects sifted from the groomee's skin or fur. The groomee commonly responded by relaxing its posture and stretching out. Social grooming could be spontaneously initiated by the groomer or solicited by the groomee. In the latter case, the soliciting animal lowered its head and shoulders in front of the other.

Sexual behaviour

Courtship and copulatory behaviour have been previously described in section 2.4d.

2.6 Behavioural observation of pairs of animals

Male-female pairs were occasionally observed in their home cages (see section 2.2) for 15-30 min. after they were placed together. Chance observations of their behaviour were also recorded (see section 2.7). However, 7 pairs were observed for a limited time in an exercise cage (see Chapter 4).

Unfamiliar combinations of male and female pairs were observed in the observation room for 15 min. at a time (i.e. the behavioural trials in Chapter 6).

2.7 Chance or casual observations of behaviour

Members of research and maintainance staff worked in the animal rooms daily between 08:00-13:00h and 14:00-17:00h (only mornings during week-ends). Observations of sexual, aggressive or infant-carrying behaviour of animals in their home or exercise cages was reported or logged in the colony records.

2.8 A computerised system for recording behaviour

The tempo of marmoset behaviour is much faster than that normally encountered in Old World monkeys and Man. Hence check

sheets and certainly written notes were awkward, cumbersome to use and led to inaccurate recording. Researchers have combatted this difficulty using several methods, such as scoring behaviour from a frame by frame analysis of cine film (Stevenson and Poole, 1976), or by dictating abbreviated codes for behaviours into a tape recorder and scoring the behaviour from a transcript (Box, 1975; Stevenson and Poole, 1976). Unfortunately, both these methods involved duplication of recording effort. Therefore, with these problems in mind, it was decided to use a behavioural event recording system that employed a 'touch type' control of keyboard switches, allowing the observer to follow the behavioural action without shifting his attention. Initially a keyboard-operated pen recording system was used (see section 2.8), but the tedium of data retrieval led to the exploration of recording behaviour in ways that a computer could understand. Some systems designed to fulfill this latter function were expensive and cumbersome (e.g. the ATSL system of Tobach, Schneirla, Aronson and Laupheimer, 1962). But Stephenson, Smith and Roberts (1970, 1976), White (1971) and Dawkins (1971) have pioneered three methods of recording behavioural data onto magnetic tape, and this information can be fed into a computer. The latter system was ultimately chosen because it did not require the relatively complex interfacing machinery of the SSR system (Stephenson et al, 1976) and WRATS (White, 1971) between the recording equipment (the electronic keyboard and magnetic tape) and the computer (however, the SSR system has great flexibility).

In the Dawkins (1971) system, keyboard switches were pressed when particular behavioural events occurred. The output from the

keyboard was recorded on an ordinary tape recorder and the tape subsequently played back into a computer. The computer eventually punched out a paper tape, registering which keys were pressed, and when, to the nearest tenth of a second. The main disadvantage of the system was that it could not record two simultaneous events. Each had to be separated by more than 18m sec.. However, a behavioural pattern which had measurable duration could be represented in terms of two recorded events, its beginning and end.

2.8a The keyboard and tape deck

The keyboard (or 'encoder') was essentially a musical electronic organ (audible only if amplified through a loudspeaker). The circuit diagram and general assembly of the keyboard are shown in Figures 2.5-2.7. The keyboard layout was re-designed and constructed from the original Dawkins' (1971) specifications. The new design was relatively simple and more reliable. A single pulse generator (Figure 2.5) was at the hub of the keyboard's function, as in Dawkins' (1971) model. Its output frequency was governed by the resistance between the base of one of the push-button keys (when it was depressed) and the voltage supply (direct current; Figure 2.6). Each push-button key brought in a different resistance and hence generated a different pulse frequency out of the generator circuit (or 'timing circuit'). The range of frequencies is given in Table 2.3. A behavioural event was recorded by pressing the appropriate key(s) once. This caused a brief train or trains of pulses of the appropriate frequency or frequencies which were recorded by the tape deck onto audio magnetic tape.

While the frequency of the signals produced by the pulse generating circuit was fixed by which key on the board was pressed,

FIGURE 2.5: Circuit diagram of the computerised system's keyboard.

KEY

RESISTOR KΩ ½W. 2%.

R_1 = 708
R_2 = 684.5
R_3 = 661
R_4 = 638
R_5 = 614
R_6 = 591
R_7 = 567.5
R_8 = 544
R_9 = 520.5
R_{10} = 497
R_{11} = 474
R_{12} = 450.5
R_{13} = 427
R_{14} = 403.5
R_{15} = 380
R_{16} = 356.5
R_{17} = 333
R_{18} = 310
R_{19} = 286.5
R_{20} = 263
R_{21} = 655
R_{22} = 10*
R_{23} = 51*
R_{24} = 1*
R_{25} = 10*
R_{26} = 270*

VR_1 = 2.2KΩ (Threshold)
VR_2 = 1MΩ (Pulse width)
VR_3 = 10KΩ (Positive amplitude)
VR_4 = 10KΩ (Negative amplitude)
SW = Switch
C_1 = 3300pF. 30V
C_2 = 0.1 μF. 160V.
C_3 = 100 pF. 30V.
C_4 = 0.1 μF. 160V.
C_5 = 0.47μF. 160V.
C_6 = 0.47μF. 160V.
C_7 = 4000μF. 25V.
C_8 = 0.22μF. 160V.
C_9 = 0.47μF. 160V.
PGC = pulse generating circuit (XR 320)
MNSTC = monostable circuit (CD 4047AE)
L = live
N = neutral
E = earth

Voltage regulator = 12V. (7812)
FUSE 1 = 200mA.
FUSE 2 = 100mA.
KEYS 1-4 = BLUE KEYS 1-4
KEYS 5-20 = GREY KEYS 1-16

All values made up using two preferred register values except those marked *.

Original drawing by courtesy of David Wight.

FIGURE 2.5

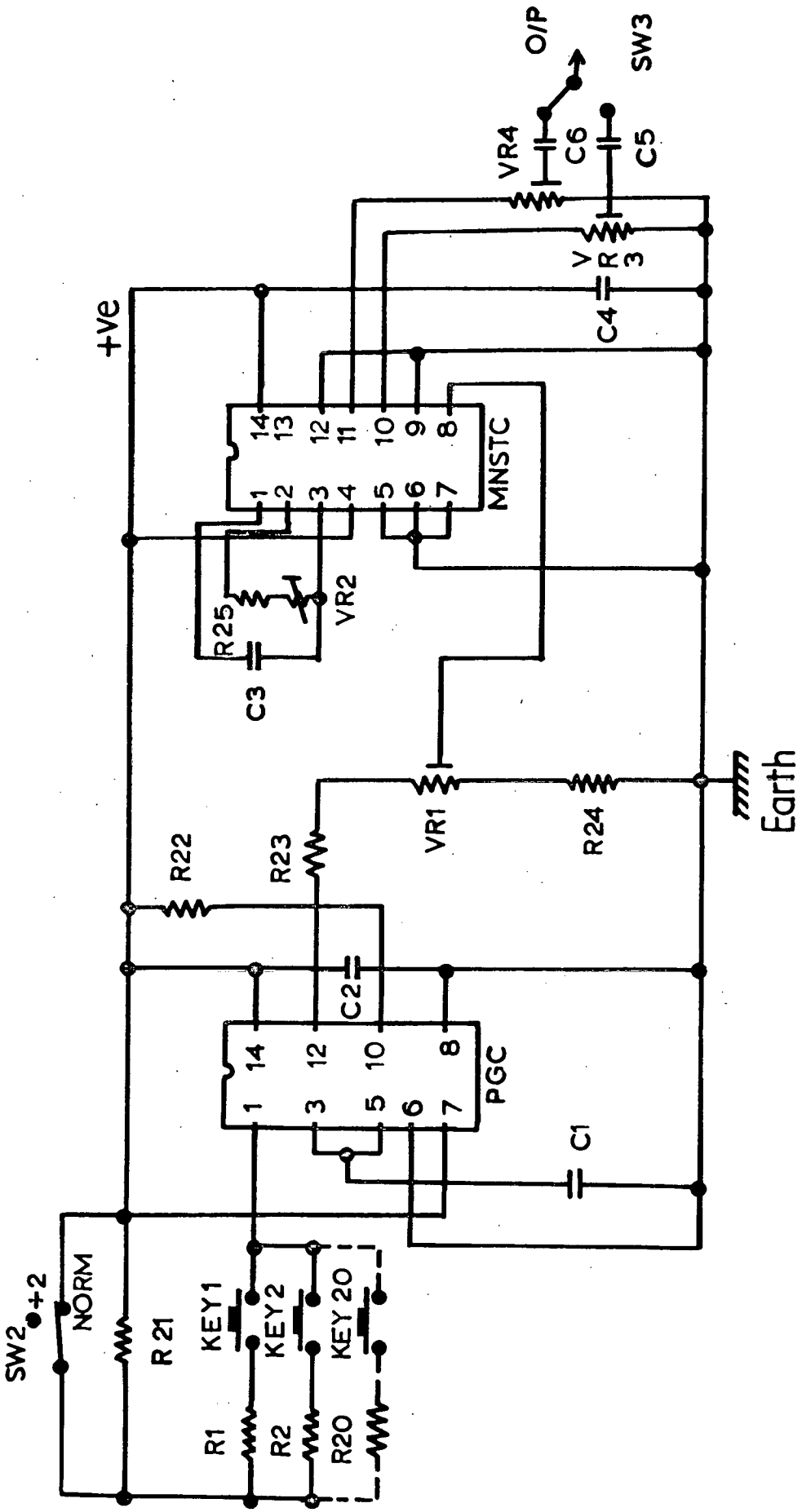
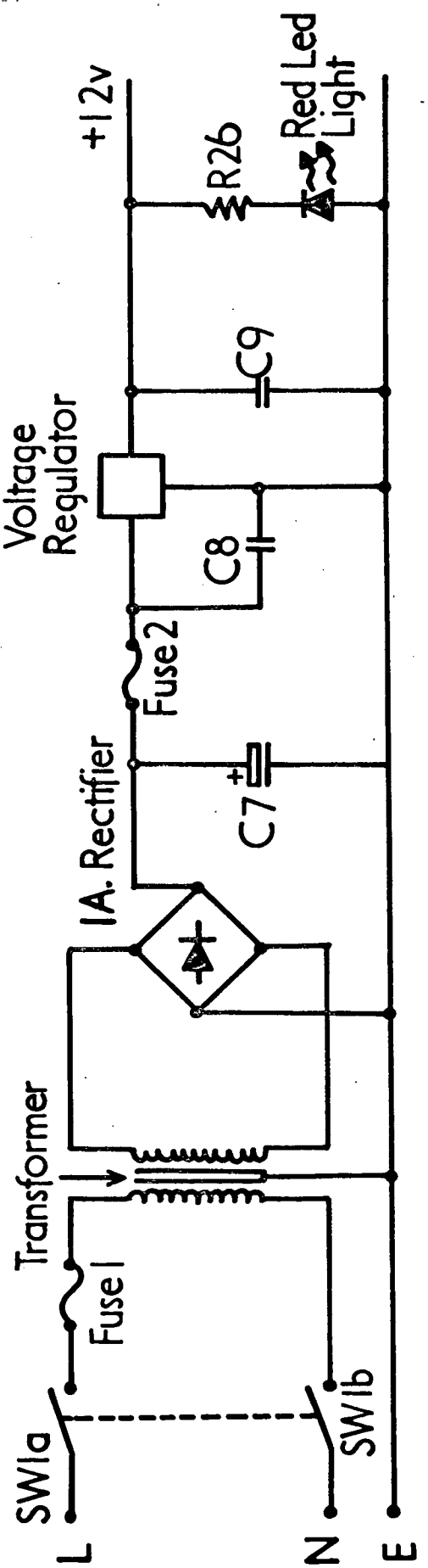

O/P
SW3
VR4
C6
C5
+ve
V R 3
C4
MNSTC
14 13 12 11 10 9 8
1 2 3 4 5 6 7
R25
VR2
C3
VR1
R24
Earth
R22
R23
C2
PGC
1 14 12 10 8
1 3 5 6 7
C1
SW2
+2
NORM
R 21
KEY1
KEY2
KEY 20
R1
R2
R20

FIGURE 2.6: Circuit diagram of the power supply to the electronic keyboard. (See the legend for Figure 2.5 for the key). Original drawing by courtesy of David Wight.



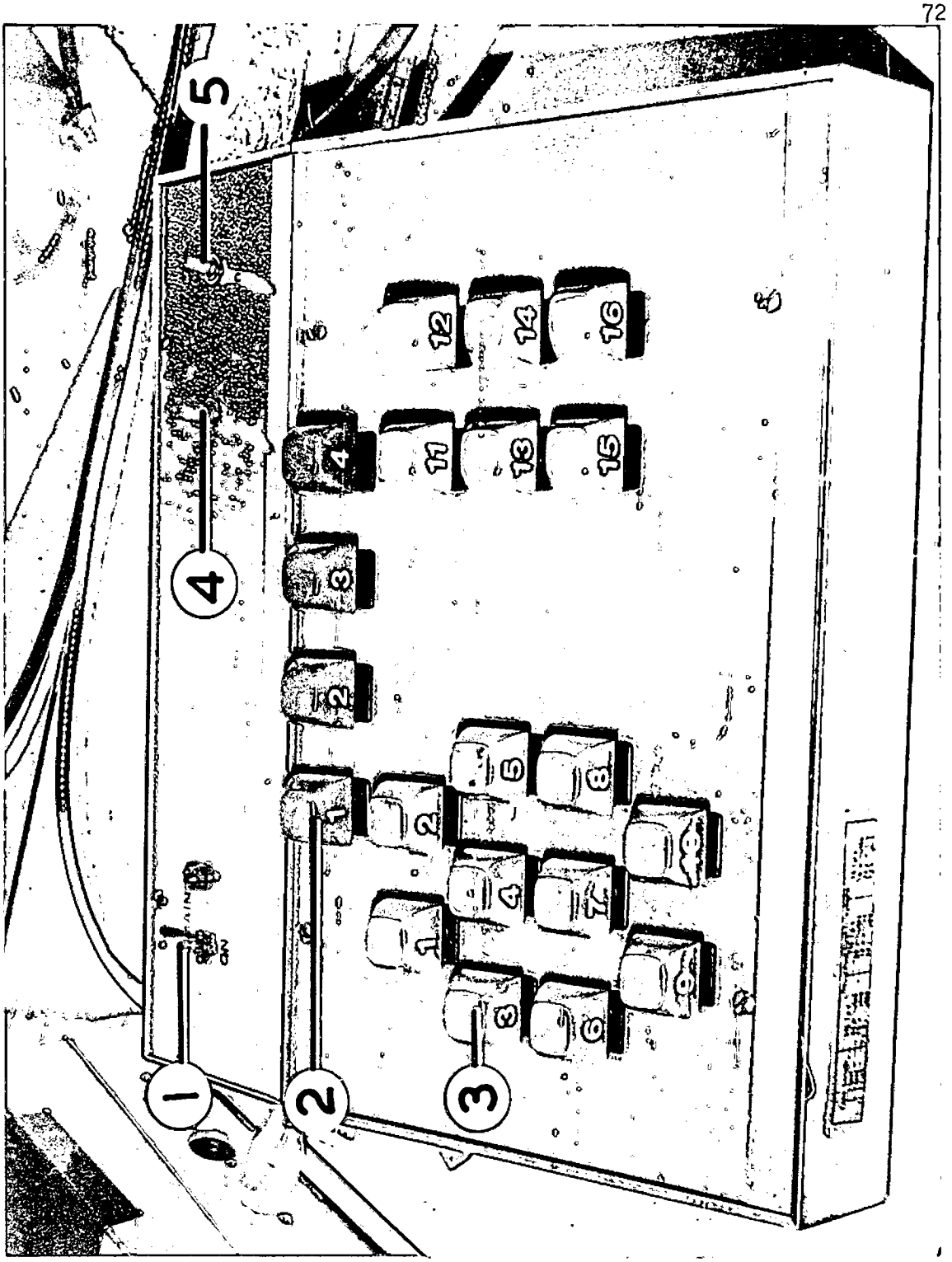


FIGURE 2.7: The keyboard and tape recorder from the computerised system.

KEY

1 = 'On'/'Off' switch

2 = Blue keys

3 = Grey keys

4 = Normal/'÷ 2' switch

5 = Positive/negative switch

FIGURE 2.13: Taking a blood sample from a hand-held marmoset receiving a dose of replacement iron syrup.

TABLE 2.3 The electrical resistance placed across each key on the keyboard and the length and frequency of the pulses produced.

KEY			'÷ 2' REGISTER		'NORMAL' REGISTER	
COLOUR	NUMBER	RESISTANCE* (KΩ)	PULSE+ LENGTH (m.sec)	FREQUENCY (CYCLES/SEC)	PULSE LENGTH (m.sec)	FREQUENCY (CYCLES/SEC)
BLUE	1	708	9.000	111	4.673	214
	2	684.5	8.845	113	4.518	221
	3	661	8.691	115	4.364	229
	4	638	8.536	117	4,209	237
GREY	1	614	8.382	119	4.054	247
	2	591	8.227	121	3.900	256
	3	567.5	8.073	124	3.745	267
	4	544	7.918	126	3.591	278
	5	520.5	7.764	129	3.436	291
	6	497	7.609	131	3.282	305
	7	474	7.454	134	3.127	320
	8	450.5	7.300	137	2.973	336
	9	427	7.145	140	2.818	355
	10	403.5	6.991	143	2.664	375
	11	380	6.836	146	2.509	398
	12	356.5	6.682	149	2.354	425
	13	333	6.527	153	2.200	454
	14	310	6.373	157	2.045	489
	15	286.5	6.218	161	1.891	529
	16	263	6.064	165	1.736	576

* Values made up using different combinations of pairs of standard resistances.

\+ See Figure 2.8 for an illustration.

the pulses had variable width (Figure 2.8). These pulses were converted by the monostable circuit into waves with a fixed pulse width (6 μ sec., regardless of frequency) to improve the quality of recording. A variable resistance was interposed between these two circuits and was tuned to cut out any circuitry 'ringing' produced by the pulse generator (Figure 2.5). The amplitude and width of pulses produced by the monostable circuit were tuned (by variable resistor 2, Figure 2.5) to produce the best possible quality of recording. This was done by monitoring the quality of the recorded pulses on an oscilloscope.

When the recorded pulses were played back and amplified, the shape of the original square waves was distorted (Figure 2.8). Every pulse now showed a positive and negative amplitude and was followed by a re-bound effect and circuitry 'ringing'. The recording was fed into the computer interface machinery and through a Schmidt trigger circuit which eliminated the re-bound effects, 'ringing' and negative amplitudes (Figure 2.8 and see below). The pulses then passed through another monostable circuit which converted the processed signals into square waves with a fixed pulse width, ready to go into the computer (Figure 2.8). As both monostable circuits were only set to fix at intervals of more than 0.3-0.4 m sec., this made doubly sure of eliminating extraneous signals (the smallest interval between pulses generated by a key on the board was 0.5 m sec.).

However, during playback some tape recorders (regardless of make) reversed the direction of amplitude of the original pulses. Thus pulses generated by the keyboard with a positive amplitude were re-emitted with a negative amplitude and vice versa. In this particular case, as the LINC-8 computer (Digital Equipment Corp.) could only

FIGURE 2.8: Conversion of the signals produced by the electronic keyboard into the signals fed into the computer.

(a) Electronic keyboard (to tape recorder).

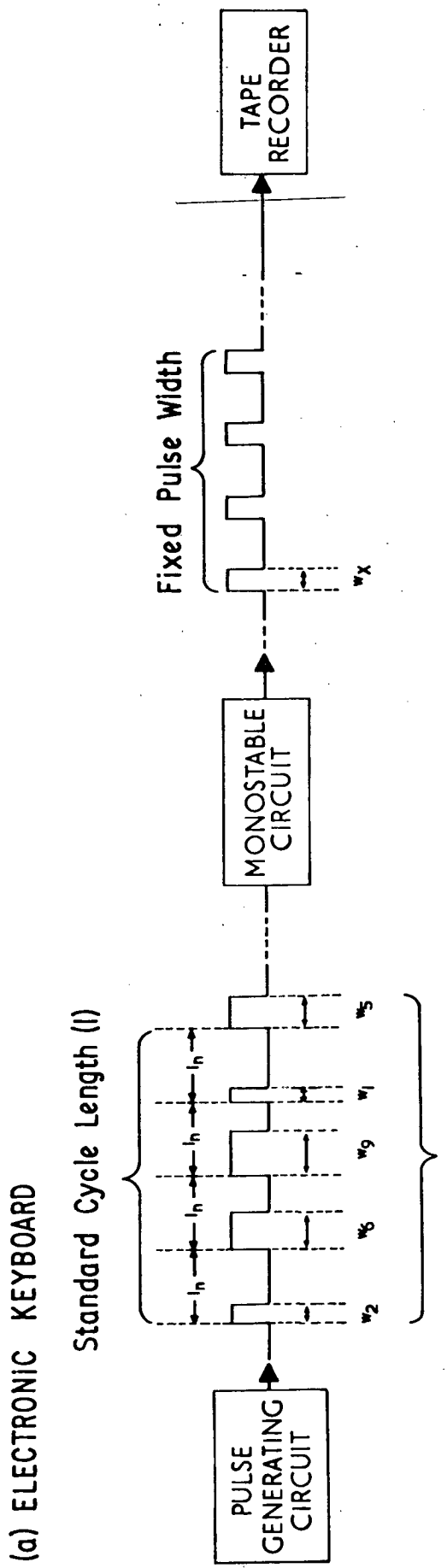
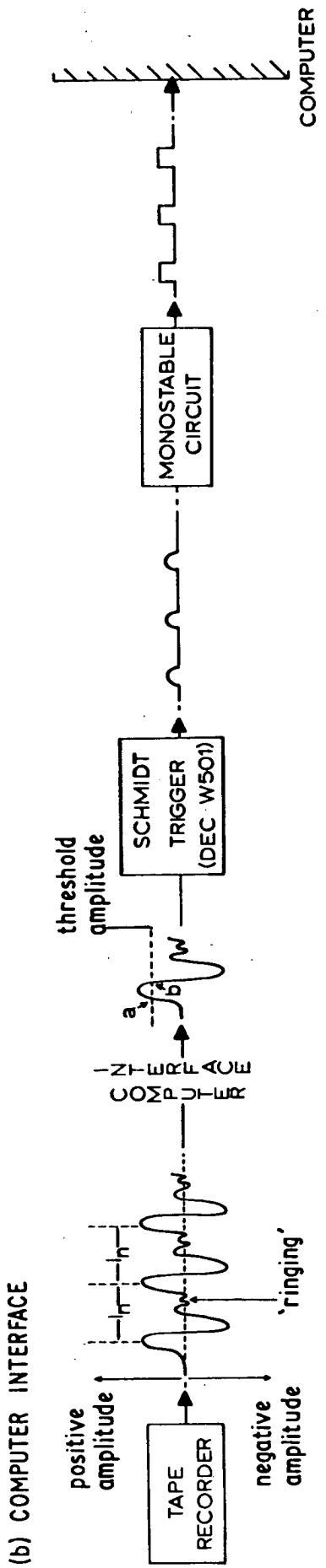


FIGURE 2.8: Conversion of the signals produced by the electronic keyboard into the signals fed into the computer.
(b) Computer interface (tape recorder to computer).



recognise positive amplitudes, each keyboard was built with the capacity to produce square waves with either positive or negative amplitude, to adapt to the idiosyncracies of each tape recorder (POS/NEG switch, Figure 2.7; SW2: C_5 (and circuit) and C_6 (and circuit), respectively, Figure 2.5).

The simplicity and low cost of this type of encoder system lay in the use of silicon-chip electronic circuits, and only one pulse generator (regardless of how many keys were on the board). The twenty keys in this case were 'normally open' push-button switches with good electrical contacts. A 'shift switch' was also used to economise on the number of keys (Figures 2.5 and 2.7). This switch interposed a low resistance in series with all the other key resistances and had the effect of dividing all the original frequencies ('normal') by two ('÷ 2'). In this way double the number of effective keys could be achieved. However, only the 'normal' frequency range was required in this study.

A good quality tape recorder was not essential for recording keyboard output, but to avoid previous distortions of the key frequencies found with some makes (P.G. Caryl and D. Wight, personal communication) a Uher 4000 Report 1C (automatic), single-track recorder was used (Audio Aids Ltd.). This is a high quality monophase recorder which can compensate for voltage reduction in the mains supply and has very little variation in its running speeds (4.7 cm./sec. in this instance). At this speed, a maximum recording time of 96 min. could be achieved on each side of tape. The speed of the tape recorder and quality of the tape were chosen to ensure minimum distortion while recording and playing back.

2.8b Encoding behaviour and animals

Using the Grey keys of the 'encoder' numbered 1 to 9 (Figure 2.7), behaviours were allocated number codings, following the example of Maurus and Pruscha (1973).

Related behaviours were grouped together, e.g. proximity and contact, play, sex, aggression and submission (Table 2.4). This eased memorizing the 'touch type' technique. Grey keys numbered 11 to 16 (Figure 2.7) were allocated to the animals observed. If any group contained more than six animals, keys 8 and 9 were also used to code for animals as '8 8' and '9 9', respectively (no group contained more than 8 animals). Key 10 was reserved for recognition by the Transcription programme and designated the start of each new line of data (see section 2.8d).

2.8c Decoding the recorded entries

The data recorded on magnetic audio tape was fed into the LINC-8 computer through a single piece of interfacing machinery (constructed and operated in the Psychology Department). Unfortunately, the tape play-back speed could not be faster than that of the original recording, because this led to errors in the computer interpretation of separate key frequencies.

The Decoding programme handled the initial analysis of the recorded data and was written in a form of machine code (a Binary programme). The programme was a modified version of that kindly supplied to Dr. P.G. Caryl (Psychology Dept.) by Dr. R. Dawkins (Zoology Dept., Oxford). This programme enabled the computer to recognise the different frequencies emitted by the different keys on the keyboard, and ordered it to punch out (on a high-speed paper tape punch) the identity of each key and the time at which it was pressed

TABLE 2.4: Numerical codes for marmoset behaviour in the computerised recording system.

NUMBER		BEHAVIOUR
FIRST KEY	SECOND KEY	
		PROXIMITY AND CONTACT:
1	1	i/t PROXIMITY
1	2	
1	3	- SOLICIT GROOMING
1	4	i/t SOCIAL GROOMING
1	5	
1	6	- SELF-GROOM*
1	7	- BODY SNIFF*
1	8	- FOLLOW
1	9	- HUDDLE
		PLAY:
2	1	i/t ROUGH -AND- TUMBLE
2	2	
2	3	i PURSUER
2	4	i/t CHASE PURSUED
2	5	
2	6	i/t HIDE -AND- SEEK
2	7	
2	8	- OPEN MOUTH
2	9	- PRONE
		AGGRESSION:
3	1	- PILOERECTION
3	2	- ARCHED-BACK
3	3	- GENITAL PRESENT
3	4	- EAR TUFT FLICK
3	5	- VOCAL THREAT
3	6	- ATTACK
3	7	-
3	8	- SCENT MARK (anal)*+
3	9	- FROWN*

NUMBER		BEHAVIOUR
FIRST KEY	SECOND KEY	
		SUBMISSION (AND SEX):
4	1	- AVOID*
4	2	- SUBMISSIVE VOCALISATION
4	3	- FACIAL GRIMACE
4	4	- FLEE
4	5	- FLATTEN EAR TUFTS
4	6	- SNIFF/LICK GENITALS*
4	7	- HEAD TURN
4	8	- LIMBS & BODY MOTIONLESS
4	9	- CROUCH
		SEX:
5	1	- INITIATE MOUNT++
5	2	- REJECT MOUNT
5	3	- TERMINATE MOUNT
5	4	- TONGUING
5	5	= ERROR; correction follows OR 6 6
5	6	- LIPSMACK
5	7	- PELVIC THRUST
5	8	- ERECT PENIS
5	9	- NUZZLE
		INFANTS, STEALING AND CHEWING:
6	1	- CLIMB ON INFANT *
6	2	- CLIMB OFF *
6	3	- TAKE PARENT OR *
6	4	- REJECT SIBLING *
6	5	- FOOD STEAL S *
6	6	- NO CORRECTION DATA
6	7	- OBJECT STEAL S *
6	8	- CHEW OBJECT *
6	9	- FEED *
7	1	- HAND *
7	2	- CUFF *

* Behaviour described in Appendix A.

\+ Classified in this category only to ease recording.

++ Denotes attempted mount if 5 1 was followed by 6 6.

S Attempt was unsuccessful if 6 5 or 6 7 was followed by 6 6.

(since the beginning of the observation). All these operations were put into action by using a programme 'interrupt' device. Normally the computer spends most of its time in a repeated time loop in which it performs no useful computation, but a count is kept of the number of cycles performed ('computer time'). Every time a pulse of electronic signals is introduced through the interfacing, the timing loop is 'interrupted'. The number of timing loop cycles since the previous interruption serves as a measure of the time interval between each interruption, and if this interval is very short, it is diagnosed as belonging within a pulse train (the group of pulses generated by pressing one of the keys on the keyboard once). Otherwise the interruption is diagnosed as the interval between two trains (e.g. when two different keys were pressed, one after the other, or the same key was pressed, twice). In either case, the length of time the interruption lasts is added to the cumulative clock time which will be eventually punched out. The 'timing loop' counter is then reset to zero and the timing loop resumed.

When the programme detects that a train of pulses has ended, it calculates the mean inter-pulse interval for that particular train. It then compares this with a complete set of 'ideal' inter-pulse intervals (corresponding to the different keys on the keyboard) played in at the start of each tape play-back. Thereby the programme establishes which key was pressed. The code for this key is punched out by the high speed punch together with the cumulative clock time of the event. Because the high-speed paper tape punch-outs are relatively time-consuming (and would otherwise interfere with the computer's handling of the input information), this output stream is temporarily buffered in the memory, and is printed out whenever there

is time between bursts of pulses. Suitable correction for this is made to the cumulative clock time punched out.

The accuracy of memory retention was tested regularly (every 3-4 months) by entering recordings of keys pressed in a fixed order (2-3 every second) for a total of 5 min. (a far greater intensity of input information than was ever generated during observations). On no occasion was any key 'forgotten' or wrongly diagnosed. However, these two mistakes did occur if the programme's mode of operation (see below) was repeatedly changed every 5-10 sec. Fortunately, this was not a relevant factor because the minimum time between changing mode during the recording of observations was approximately 1 min.

The Decoding programme operated in four different modes: 'Calibration', 'Text', 'Event recording' and 'Analogue'. The latter three modes were designated by the first three blue 'command' keys on the keyboard (Blue 1-3, respectively; Figure 2.7). The keyboard carried a fourth 'command' key which could be used to designate a further mode of operation, but during this study it was only translated as a single blank space.

2.8c(i) Calibration mode

In this mode the computer memorized the inter-pulse intervals for every key on the keyboard. Each key was pressed once, starting with Blue 1-4 and continuing with Grey 1-16 (effectively 'playing a scale'). The Decoding programme automatically set the computer into the Calibration mode at the start of each play-back session (a 'scale' was recorded before the start of every observation). In this way, any slight alteration in the frequency generated by any key could be easily accommodated.

When the 'scale' was finished, the first key pressed during Calibration (in this case, Blue 1) was pressed again and the programme left the Calibration mode to recognize the frequencies which would occur during the rest of the tape.

2.8c(ii) Text mode

When the Decoding programme left the Calibration mode it entered the Text mode (designated by the command key Blue 1). In this mode the grey keys corresponded to a letter of the alphabet, a figure, or a punctuation mark (Table 2.5). Hence each observation could be labelled with an experiment title, dates, animal numbers, and so on. No time information was printed in this mode and the eventual print-out (after Transcription, see Section 2.7d) resembled that of an ordinary typewriter (e.g. Figure 2.9).

2.8c(iii) Event Recording mode

On completion of the text entries, the programme was told to leave the Text mode and enter the Event Recording mode by the pressing of command key Blue 2 (the second key on the calibration scale). When this was done the cumulative clock time counter was set to zero. If any grey key was pressed a new line was designated, the cumulative clock time (in tenths of seconds) was punched out and was followed by the symbol of the key in this mode (numerals 1-16, as labelled on the keyboard; Figure 2.7). An example of a sequence of this data is shown in Table 2.6. All behavioural recordings were decoded in this mode.

The Decoding programme was returned to the Text mode to signify the end of each observation (by pressing command key Blue 1). The label of the next animal to be observed was then fed in and the

TABLE 2.5: The corresponding symbol for each grey key on the electronic keyboard during the Text mode.

GREY KEY	SYMBOL
1	1
2	2
3	3
4	4
5	5
6	6
7	7
8	8
9	9
10	0
11	T
12	F
13	P
14	-
15	*
16	M

EXPERIMENTAL TREATMENT (IF ANY): TESTOSTERONE IMPLANT

'TARGET' ANIMAL: THE FEMALE CO-TWIN WITHOUT THE SHAVED TAIL (No.13)

	FAMILY NO. 12	DATE	AGE OF THE 'TARGET' ANIMALS (DAYS)			
TITLE OF THE OBSERVATION.	F 12	- 15-11-76	- 250	- TIMP	- F	-

THE TIME AT THE START OF AN EVENT (TENTHS OF A SECOND)					'ERROR' SIGNAL FOLLOWED BY CORRECTION			
82:	13	12	1	1				
222:	12	13	1	2				
287:	14	13	2	1				
310:	13	14	2	3	5	5	2	6
417:	14	13	2	1				
467:	13	14	2	2				
546:	13	14	2	6				
584:	13	14	2	7				
717:	14	13	1	1				
735:	14	5	5	13	14	1	2	
769:	13	15	1	1				
794:	15	13	1	2				
958:	13	15	2	1				
1087:	13	15	2	2				
1175:	16	13	2	1				
1198:	16	13	2	2				
1239:	13	13	1	6				
1467:	13	7	14	15	2	1		
1483:	14	15	7	13	2	2		
1597:	16	13	2	1				
1627:	14	7	16	13	2	1		
1663:	13	14	7	16	2	2		

FIGURE 2.9: Transcribed behavioural data processed by the computer. The exert is the first 2-3 minutes observation of a female marmoset (implanted with testosterone neonatally) in her family group.

TABLE 2.6 Print out of some data processed by the decoding programme.

TIME SINCE THE START OF THE OBSERVATION (TENTHS OF A SECOND)		KEY SYMBOL	COMMANDS AND MODE OF OPERATION
14	-	Z	- EVENT RECORDING COMMAND
87		10	EVENT RECORDING MODE
93		16	
97		15	
101		1	
106		2	
451		10	
456		15	
459		16	
463		1	
468		1	
585	-	A	- ANALOGUE COMMAND
587		568	ANALOGUE MODE
590		438	
593		372	
597		855	
602		853	
635		570	
641		436	
645		371	
650		856	
655		855	
663	-	T	- TEXT COMMAND
FFFF			- END SEQUENCE

programme returned to the Event Recording mode. If no further data was to be recorded after the completion of an observation, the letter 'F' was recorded four times (while in the Text mode) indicating that the whole input from the observation was ready for Transcription (Section 2.7d).

2.7c (iv) Analogue mode

This mode was seldom operated during this study. It was selected by pressing the third key pressed during calibration (Blue 3). In effect it disabled the frequency recognition routine. When a train of pulses was detected in this mode, the mean inter-pulse interval was not compared with the 'scale' played in during the Calibration mode, but was punched out as raw data complete with the clock time at which the train of pulses was 'heard'. The inter-pulse intervals were punched out as 'timing loop units' (units of 'computer time', i.e. the mean number of times the programme cycled round the timing loop between interruptions). This measurement was inversely proportional to the key frequency.

The data output was therefore minimal (Table 2.7) and was routinely used to check the frequencies of each key. In terms of 'computer time', the latter varied very little. The information obtained in this mode could serve to represent the distance between two courting animals, and so on.

2.8d Transcription of recorded behaviour

Following the decoding of an observation, the Transcription programme was inserted (written by Dr. P.G. Caryl in PSYFOC, a local version of the DEC-FOCAL computer language). The roll of punch tape generated by the decoding process was then fed through

TABLE 2.7 The frequency-related value of each key in the analogue mode.

KEY		FREQUENCY-RELATED VALUE (UNITS OF 'COMPUTER TIME') +	
		NORMAL	÷ 2
BLUE	1	985	1880
	2	930	1845
	3	A‡	1816
	4	890	1781
GREY	1	855	1750
	2	825	1716
	3	795	1689
	4	761	1653
	5	729	1623
	6	695	1591
	7	664	1559
	8	634	1528
	9	602	1496
	10	568	1461
	11	534	1433
	12	502	1398
	13	471	1369
	14	438	1334
	15	405	1303
	16	372	1270

+ See text.

‡ Analogue command key.

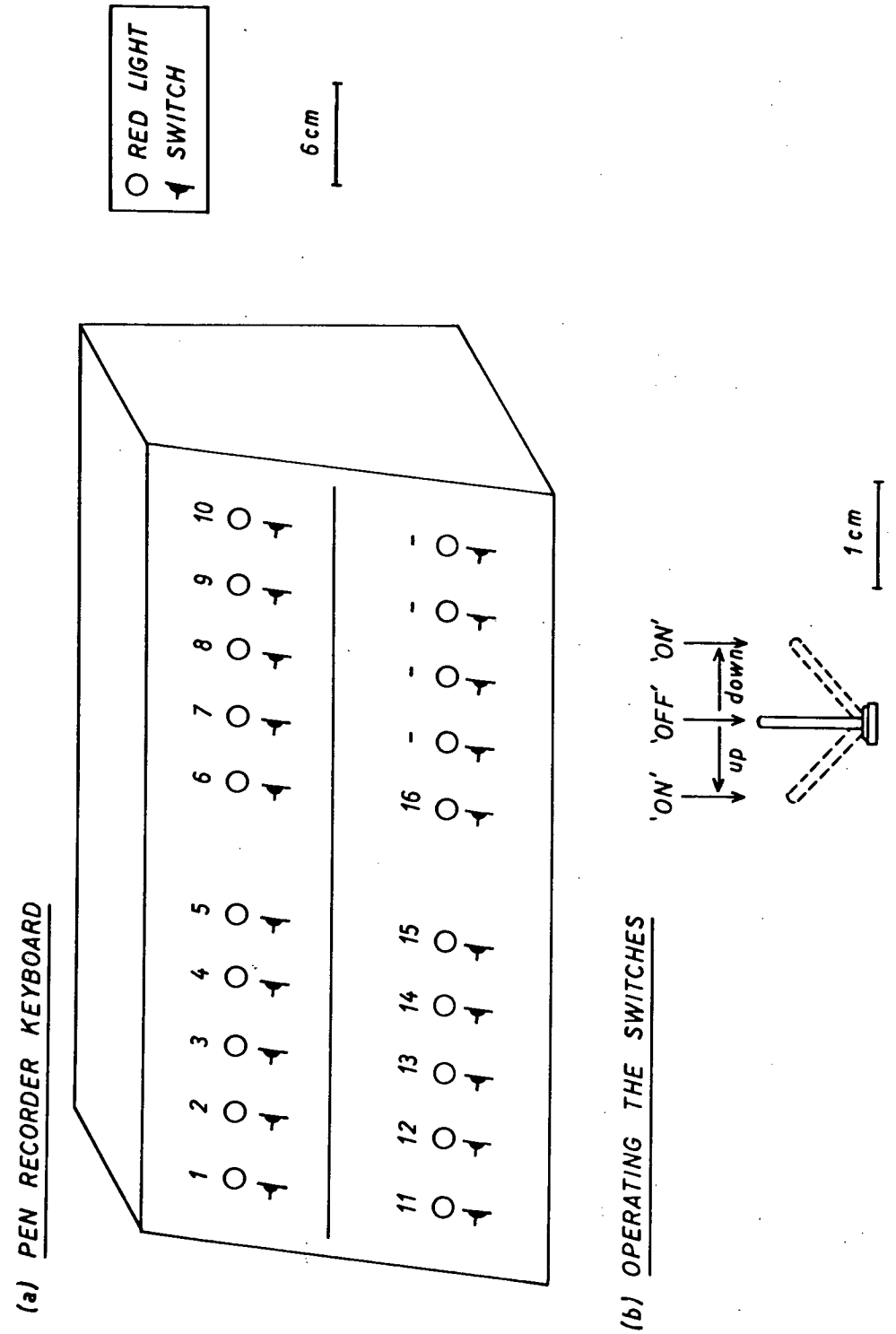
the high-speed punch tape reader and the data was printed out on the line-printer (e.g. the data print-out in Figure 2.9). When transcribing data punched out in the Event Recording mode of the previous programme, the Transcription programme searched for the code of Grey key 10. When this was found a new line was started, the time at which the key was pressed was printed, the programme deliberately 'forgot' to print out '10', and printed out the numbers of all the other Grey keys pressed (without their corresponding times) before key 10 was pressed again. In this way each behavioural interaction between two or more animals (and the time at which it occurred) was easily identified on a separate line from any other data. For example, take the first two lines of recorded behavioural data in Figure 2.9 . The first animal (13) delivered a behaviour (designated at the right-hand end of the line) at the second animal (12). In this case animal 13 initiated proximity ($\leq$ 10cm.) to animal 12, 8.2 sec. after the start of the observation (the time is shown at the left end of the line, followed by a colon). On the next line, 22.2 sec. after the start of the observation, animal 12 left the proximity of animal 13 (a duration of 5.5 sec). If more than one animal initiated and/or terminated a behaviour, a figure '7' was inserted in the input of data between the two groups of animals, e.g. the rough-and-tumble play sequence in Figure 2. 9. If two animals initiated and/or terminated a behaviour towards each other simultaneously, the number '7' was repeated twice at the end of that line, e.g. Figure 2. 9.

Frequencies of behaviours were easily scored from the print-outs and stored on charts for each family, group or pair. No further computer manipulation was required.

2.9 A multi-pen recorder system for behaviour

Prior to the computerised recording system becoming fully operational in September, 1976, an Esterline Angus pen recorder was used during behavioural observations. A simple keyboard (Figure 2.10) was designed to operate the pens on the recorder and contained twenty flip switches (one for each of the pens on the Esterline Angus). Each switch could be put into the 'on' position by pushing it up or down. This deviated the relevant pen by 2-3 mm. from its normal path, as illustrated in Figure 2.11. When pushed upwards, every switch remained 'on' (illuminating the red light above it) until the observer pulled it back to the central 'off' position (Figure 2.10). When pushed downwards into the 'on' position, each switch automatically flipped back to the 'off' position when released by the observer (the red light above each one did not light up during this mode of operation). The first mode of switch generation was employed to record behaviours of long duration (the red light serving as a reminder that a particular switch was 'on'). The latter mode was used to record rapid activity and only this mode was operated when the Esterline-Angus paper traces were made compatible with the computerised system. By doing this, observations could be recorded months before the computerised system became operational and the information stored on Esterline Angus paper traces. These traces were then re-played on the pen recorder (minus pens) at a later date and recorded on the modified Dawkins system. The switches on the Esterline Angus keyboard were numbered identically to the Grey keys on the 'Dawkins' keyboard (Figure 2.10 and 2.7, respectively) and an example of a recorded paper trace is shown in Figure 2.11 with its computerised equivalent.

FIGURE 2.10: The keyboard operating the Esterline Angus pen recorder.



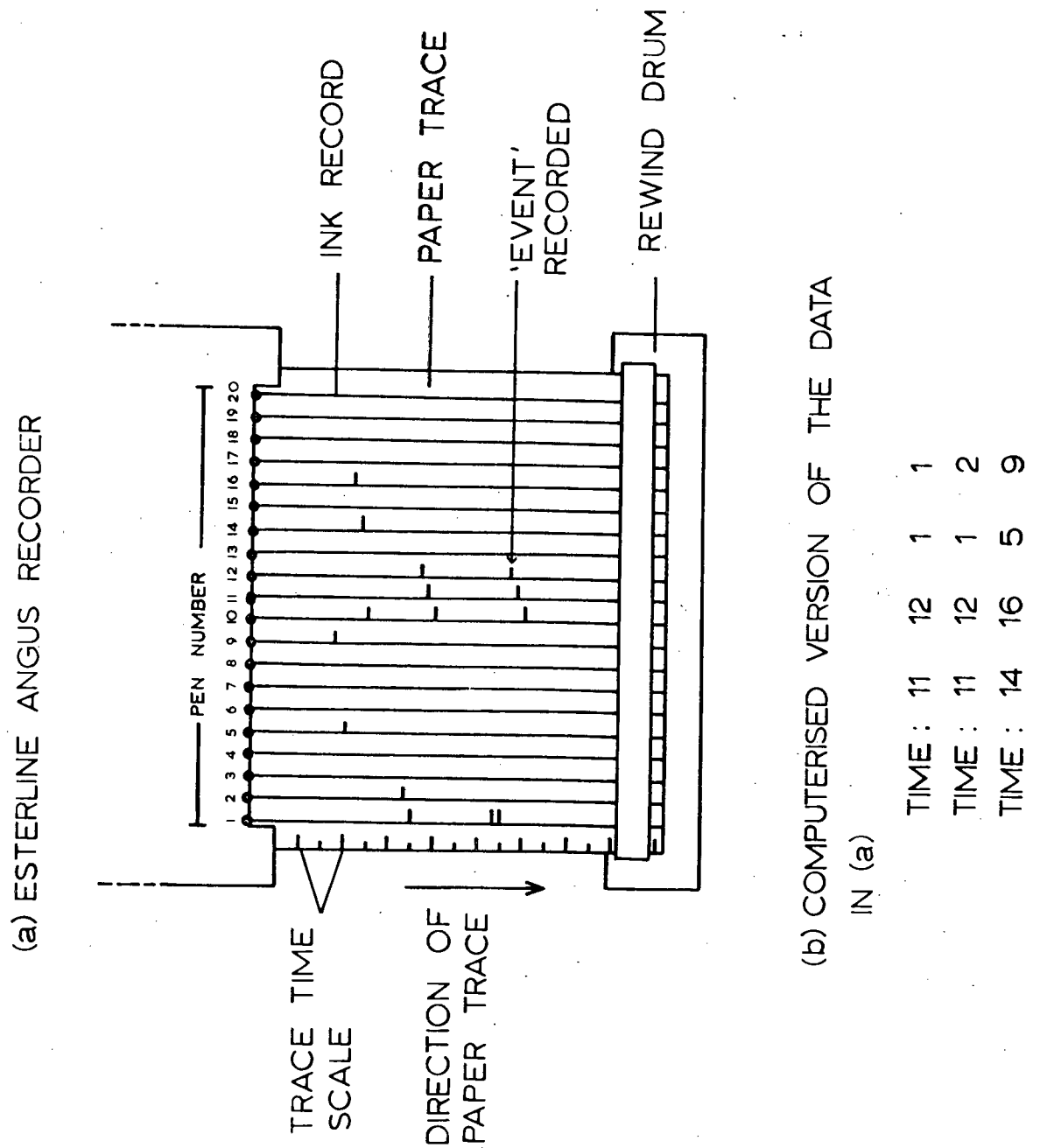


FIGURE 2.11: An exerpt from behavioural data recorded on the pen recorder system and its computerised equivalent.

2.10 Recording behaviour with abbreviated notes

When peer groups were observed in exercise cages, their behaviour was recorded in the form of abbreviated notes. This method was sufficiently expedient to permit little distraction from actual recording, but did produce voluminous sheefs of data without any accurate timing. This method was necessary because the animals were observed without using a hide and excessive movements, such as those required to change tapes on the tape recorder of the computerised system, disturbed the monkeys (observation sessions lasted longer than the 96 min. of each side of tape). The bulky pen recorder was also impractical in this situation.

Both animals and behaviours were denoted by letters of the alphabet and an example of date entries is shown in Table 2.8. . The time was noted every 15 min.

2.11 Vaginal washings and the detection of inseminations

The cervical and vaginal region of female marmosets was flushed with 0.1ml. sterile physiological saline which was introduced to the vagina by a 1 ml. syringe with a 0.81 mm. diameter (21 gauge) needle tipped with a 5 mm. length of transparent plastic tubing (Hearn et al, 1975). A 1 ml. eppendorf pipette tip, cut at its end to a size that allowed passage of the plastic tubing, was first inserted into the vagina as a speculum. The needle with its plastic tip was gently inserted through this so that washings did not come into contact with the external genitalia. The animal was restrained in the same way as for bleeding (section 2.12) while the legs were held apart (e.g. Figure 2.12).

The washings were examined under a microscope (x100 magnification) within 2-3 min. of sampling. Initially the washings were observed

TABLE 2.8: Abbreviations used (a) while recording the behaviour of marmosets in a peer group and (b) in a short exerpt from recorded peer group behaviour.

(a)

BEHAVIOUR	ABBREV-IATION	BEHAVIOUR	ABBREV-IATION
Aggression:		Sexual:	
vocal threat	VA	mount	M
ear tuft flick	ETF	reject mount	REJM
genital present	GP	accept mount	ACCM
attack	AT	attempt mount	ATTM
pursue	P	terminate mount	FINM
Submission:		pelvic thrusting	T
submissive vocalisation	SV	tonguing	TI/O
facial grimace	GR	lipsmack	LS
flee	FL	erect penis	PEER
flatten ear tuft	ETD	head turn	HT
Social:		limbs and body motionless	LBM
proximity (initiated)	⟶	crouch	C
huddle	HUD	Displays:	
follow	FOL	piloerection:	
nuzzle	NUZ	- without arched-back	HB
social groom	SOG	- with arched-back	HB

(b)

EXPANDED FORM	ABBREVIATED NOTE
White-red submissively vocalised and grimaced at red-red. Red-red threatened white-red.	WR SV GR RR VA
White-white initiated proximity to green-green.	WW ⟶ GG
Red-white tongued and lipsmacked at white-white while following her (pursuit courtship)	RW TI/O LS FOL WW

FIGURE 2.12: Taking a vaginal lavage.

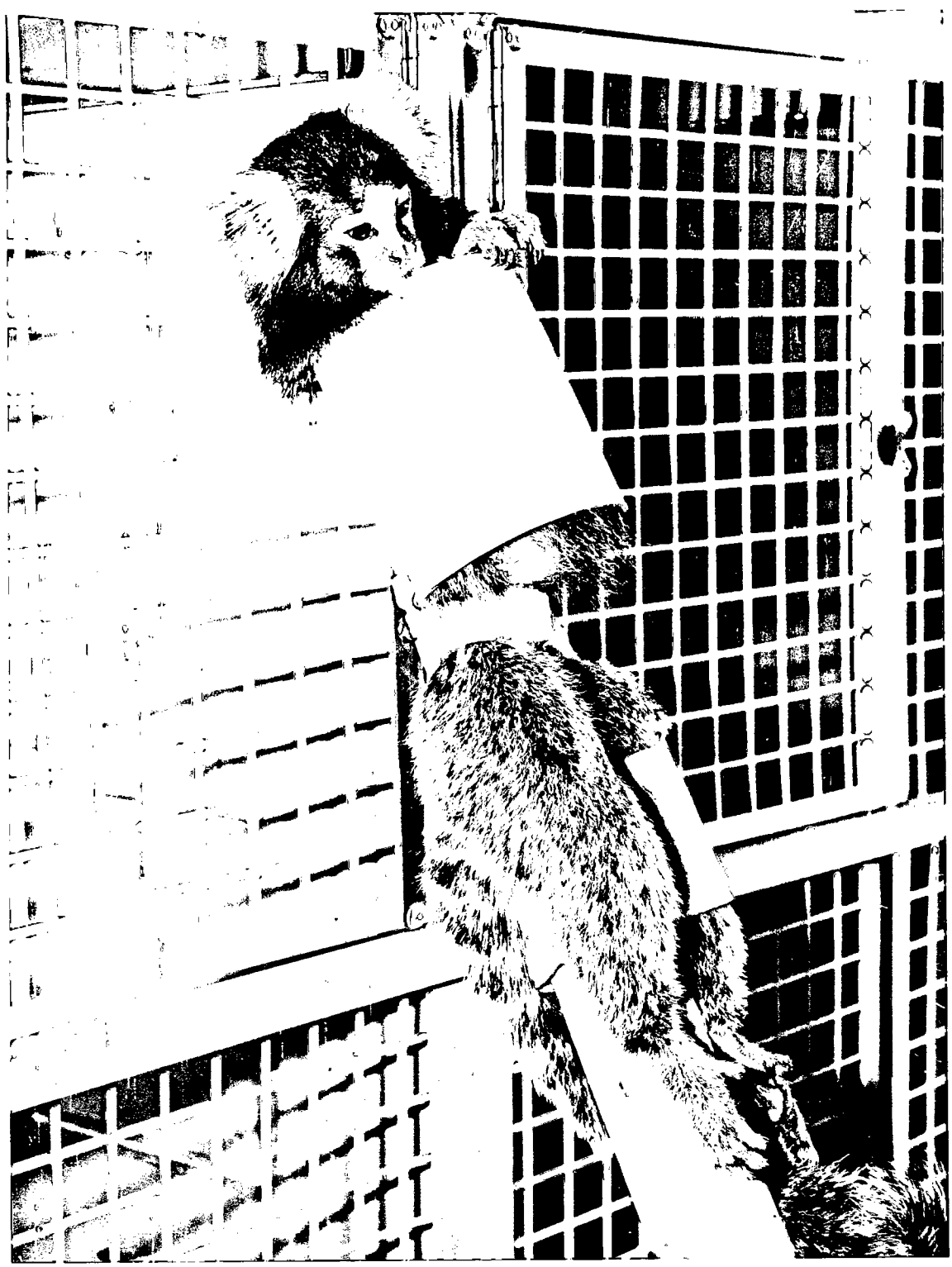


FIGURE 2.14: A marmoset in the restraining device.

under phase contrast for any motile spermatozoa and the whole sample was scanned. If a permanent record was required, the sample was left to dry on the glass microscope slide and then dipped into a solution of methylene blue for 5 min. Any detailed scanning of the stained spermatozoa was carried out at x 400 magnification.

2.12 Collection of blood

Blood samples were taken from the femoral vein using a 0.41 mm. diameter (27 gauge) needle and a heparinised 1 ml. syringe, and placed immediately on ice. The syringe was sealed with a steriseal cap, centrifuged at 2500 rpm (500 g) for 20 min. at 4°C, and the plasma stored at -20°C. until assayed. Animals were either restrained manually by a handler (Figure 2.13; Hearn et al, 1975), or in a restraining device that allowed a single person to manipulate the monkey and collect the blood sample (Figure 2.14; Hearn, 1977).

When either system was employed, the marmosets remained relaxed and appeared to be under little stress. After collecting the blood sample, animals were given 0.2 ml. of iron syrup ('Fersamal', Glaxo Laboratories Ltd.) as a reward and for replacement of iron (e.g. Figure 2.13).

2.13 Radioimmunoassay for progesterone

Plasma progesterone was measured by a radioimmunoassay method similar to that described by Scaramuzzi, Corker, Young and Baird (1975) and Neal, Baker, McNatty and Scaramuzzi (1975). The system utilised an antiserum (number 343 (7), donated by Dr. K. Dighe) which was raised in a rabbit against progesterone-11α-hemisuccinate-bovine serum albumen conjugate. The specificity of the antiserum has been previously tested by Dighe and Hunter (1974). The assay was adapted

to deal with the small samples of plasma obtainable from marmosets; consequently the procedural details are given here.

2.13a Reagents

All reagents used were Analar grade from BDH (unless otherwise stated).

Assay buffer : 0.05M. phosphate gelatin buffer

36gm. of sodium chloride, 34.4gm. of disodium hydrogen orthophosphate, 24.32gm. of sodium dihydrogen orthophosphate and 4gm. of gelatin were dissolved in 4l. of distilled water. 0.04gm. sodium thiomersalate (0.001% $^{w}/_{v}$)was added as a preservative.

Solvents : Analar grade petroleum ether (B.P. 40-60°C.) and Aristar grade ethanol (BDH) were used.

Stripping agents : Activated charcoal Norit A was obtained from Sigma and dextran T70 was supplied by Pharmacia Fine Chemicals.

Steroids : Non-radioactive progesterone was obtained from Sigma and accurately weighed amounts were dissolved in ethanol and stored at 4°C.

Radioactive progesterone (1,2,6,7-^{3}H-progesterone, 284 µCi/µg. (250 µCi in 0.25ml. benzene; 0.88 µg.); Radiochemical Centre, Amersham) was diluted to a concentration of 125 µCi (0.44µg)/ml. in ethanol and stored at 4°C. For use in the assay, 50 µl. (22 ng) of the stored tracer was evaporated and the residue dissolved in 40 ml. of buffer to give approximately 10,000 cpm/100 µl (55pg/100µl).

Scintillation fluid : This was prepared by adding 10gm. of 2,5-diphenyl-oxazole (PPO; Koch-light) and 750mg. of p-tris (2-(2 phenyl-oxazolyl))-benzene (POPOP; Koch-Light) to 2.5l. of toluene (analytical grade, Koch-Light). 1.25l. of Triton X-100

(analytical grade, Koch-Light) were also added and the mixture stirred until a homogenous solution was obtained. 10 ml. of this fluid were added to each scintillation vial (plastic disposable, New England Nuclear) before counting, using a semi-automatic dispenser (30 ml. capacity Zipette; Jencons).

Disposable glass tubes and eppendorf pipettes with disposable plastic tips were used throughout the assay. The anti-serum and tracer were dispensed with 2 ml. capacity repettes (Jencons) and petroleum ether added using a semi-automatic dispenser (Lumix; Chem. Lab. Instruments Ltd.).

2.13b Assay procedure

2.13b(i) Extraction of progesterone from plasma

20 or 50 µl. duplicate aliquots of plasma were pipetted into glass extraction tubes (75 x 16 mm.; Gallenkamp) and phosphate gelatin buffer added to produce a total volume of 100 µl. 1.0 ml. of petroleum ether (distilled not more than 24 hours previously) was added and the tubes mixed vigorously in a multivortex mechanical shaker (Baird and Tatlock) for three minutes (the time taken to achieve maximum recovery of progesterone; Clarke, 1976). When the aqueous and solvent phases had separated out, the aqueous phase was frozen quickly by placing the tubes into ethanol containing dry ice. The solvent phase was then decanted into 75 x 12 mm. glass tubes (Kimble: Corning). These tubes were placed in a heated block at 45°C. (Driblock DB3, Tecam) and the solvent evaporated to dryness under a regulated flow of nitrogen.

2.13b(ii) Radioimmunoassay

The dried residue from the extraction procedure was re-dissolved in 0.3 ml. of buffer. The contents of the tubes were mixed thoroughly

on a vortex mixer and were either left overnight at 4°C (approximately 16 hours) or left to stand at room temperature for at least 3 hours. The contents of the tubes were mixed for a second time, after which duplicate aliquots of 100 µl of the solution were transferred to glass assay tubes (75 x 10mm.; Kimble: Corning). 50 µl. aliquots were transferred simultaneously to counting vials for recovery determination (see below).

For each assay a standard curve was constructed using progesterone standards which had been dissolved in ethanol and diluted in buffer. Standards were serially diluted and aliquotted in duplicate so that tubes contained 31.25 - 1000 pg of progesterone in a volume of 100 µl. 100 µl of antiserum in buffer (initial dilution 1:10,000) and 100 µl of 1,2,6,7-^{3}H-progesterone in buffer (55 pg.) were added to all tubes containing standards and unknowns, to give a final incubation volume of 300 µl. In addition, duplicate sets of total counts (TC), non-specific binding (NSB) and total bound (TB) tubes were set up as follows:-

TC	:	^{3}H-progesterone	(100 µl)
NSB	:	^{3}H-progesterone	(100 µl)
		buffer	(200 µl)
TB	:	^{3}H-progesterone	(100 µl)
		antiserum	(100 µl)
		buffer	(100 µl)

Tubes were either incubated overnight at 4°C or at room temperature for at least 4h., and then placed on ice. Meanwhile a suspension of dextran-coated charcoal in buffer (25mg. dextran and 250mg. charcoal per 100ml. of buffer). 1.0ml. of this suspension (kept on ice and continuously stirred) was added to all tubes, except

the TC tubes which received 1.0ml. buffer. The tubes were mixed and left on ice for 15 min. All tubes (except those labelled TC) were centrifuged at 2500 rpm for 10 min. at 4°C., and the supernatants immediately decanted into counting vials. Following the addition of 10 ml. of scintillation fluid, the vials were allowed to equilibrate in a cooled (4°C) scintillation counter (β-particle radiation detector; Packard, Model 3375) for 30-60 min. before counting.

2.13b(iii) Recovery

The recovery of hormone from plasma was calculated for each sample during the first 13 assays. Thereafter, single recoveries were determined for 25 of the duplicate aliquots of plasma from samples included in the assay. A mean recovery was then applied to all samples in the assay. In the initial 13 assays, mean recoveries ranged from 61.4 - 78.4% and the coefficient of variation for recovery between samples within an assay varied between 4.42 and 11.20%.

The amount of progesterone extracted from plasma was estimated by adding 20 µl of 1,2,6,7-^{3}H-progesterone in ethanol (11pg, approximately 2000 cpm) to the plasma/buffer mixture (100 µl) in 75 x 16mm. glass extraction tubes. The contents of the tubes were immediately mixed on a vortex mixer and left at room temperature for 15 min. Extraction and evaporation to dryness were carried out as described for the assay. Following the addition of 300 µl of buffer to the residue, the tubes were mixed and were either placed in the fridge overnight (4°C) or left to stand at room temperature. 50 µl. aliquots of this solution were then transferred to scintillation vials which were counted as before. Two 20 µl. aliquots of the tracer (total recovery counts) were also counted.

2.13c Inter- and intra-assay variation

The precision of the assay was assessed by repeated assay of quadruplicate aliquots from a pool of human female plasma (Quality controls). A mean ($\pm$ S.E.M.) value of 11.13 $\pm$ 0.21 ng/ml. was obtained with a coefficient of variation of 14.93% as a measure of inter-assay variation (n=13). A value of 10.70 $\pm$ 0.18 ng/ml. was obtained with a coefficient of variation of 5.82% for intra-assay variation (n=12).

2.13d Sensitivity

The limit of detection of the assay (B/B_o = 90%) was 15 $\pm$ 2 pg/tube (mean $\pm$ S.D.; Chambers and Hearn, in press). With a 20 µl plasma sample the detection limit was therefore 2.1 ng/ml (assuming a 70% recovery).

2.13e Calculations

The construction of a standard curve and the determination of the amounts of progesterone in plasma samples was accomplished with the aid of a programme written by Dr. R.M. Sharpe for use in a desk computer (9821A Calculator; Hewlett Packard). The programme was written to construct a straight line from the typically sigmoid standard curve. A standard curve was calculated using a Y-axis of the logit transformation of B/Bo (where B = counts in the standard tubes - counts in the NSB tubes, and Bo = counts in the TB tubes - counts in the NSB tubes) and an X-axis of the pg values of the standards on a log scale at a dose interval of 2. Values for logit B/Bo versus dose of standard were plotted by the computer. The calculations of a standard curve omitted the points $>$ 90% of Bo and $<$ 10% of Bo, and a straight line of best fit for the data points was

drawn. Progesterone concentrations were calculated by the computer as pg/tube and were later corrected to give ng/ml plasma. A typical standard curve obtained by this procedure is shown in Figure 2.15.

2.14 Radioimmunoassay for oestradiol-17β

The radioimmunoassay for oestradiol-17β was similar to that described for progesterone, and full details of the methodology and specificity of the assay have been described by Baird, Swanston and Scaramuzzi (1976). The antiserum (OR-422/7) was raised in sheep injected with oestradiol-6-keto-bovine serum albumen. Cross-reactions tested with other steroids included:- 6-keto-oestradiol (100%), oestrone (9.6%), oestradiol-17β (2.8%) and other steroids (< 1%). The assay for oestradiol-17β differed from that for progesterone in the following ways:-

Non-radioactive oestradiol-17β (Sigma) was stored in ethanol at 4°C. at a concentration of 1µg/ml. 512µl. were removed, evaporated to dryness and the residue dissolved in 100ml. of buffer. Suitable dilutions of this solution were then prepared to give concentrations of 512, 256, 128, 64, 32, 16 and 8 pg/100 µl of buffer. These were used as standards.

Radioactive oestradiol-17β (6,7-^{3}H-oestradiol-17β) with a specific activity of 156 µCi/ug (250 µCi (1.6µg) in 0.25ml. benzene-ethanol, 9:1) was obtained from New England Nuclear and stored at 4°C. in ethanol (125 µCi (0.8µg.)/ml.). In the assay, approximately 6000 cpm of 6,7-^{3}H-oestradiol-17β (i.e. 100pg) per 100µl. buffer were added to each tube.

To test the recovery of oestradiol-17β from plasma (100µl aliquots), 20µl of 6,7-^{3}H-oestradiol 17β in ethanol (approximately 1200 cpm) were added and extracted in a synonymous way to progesterone.

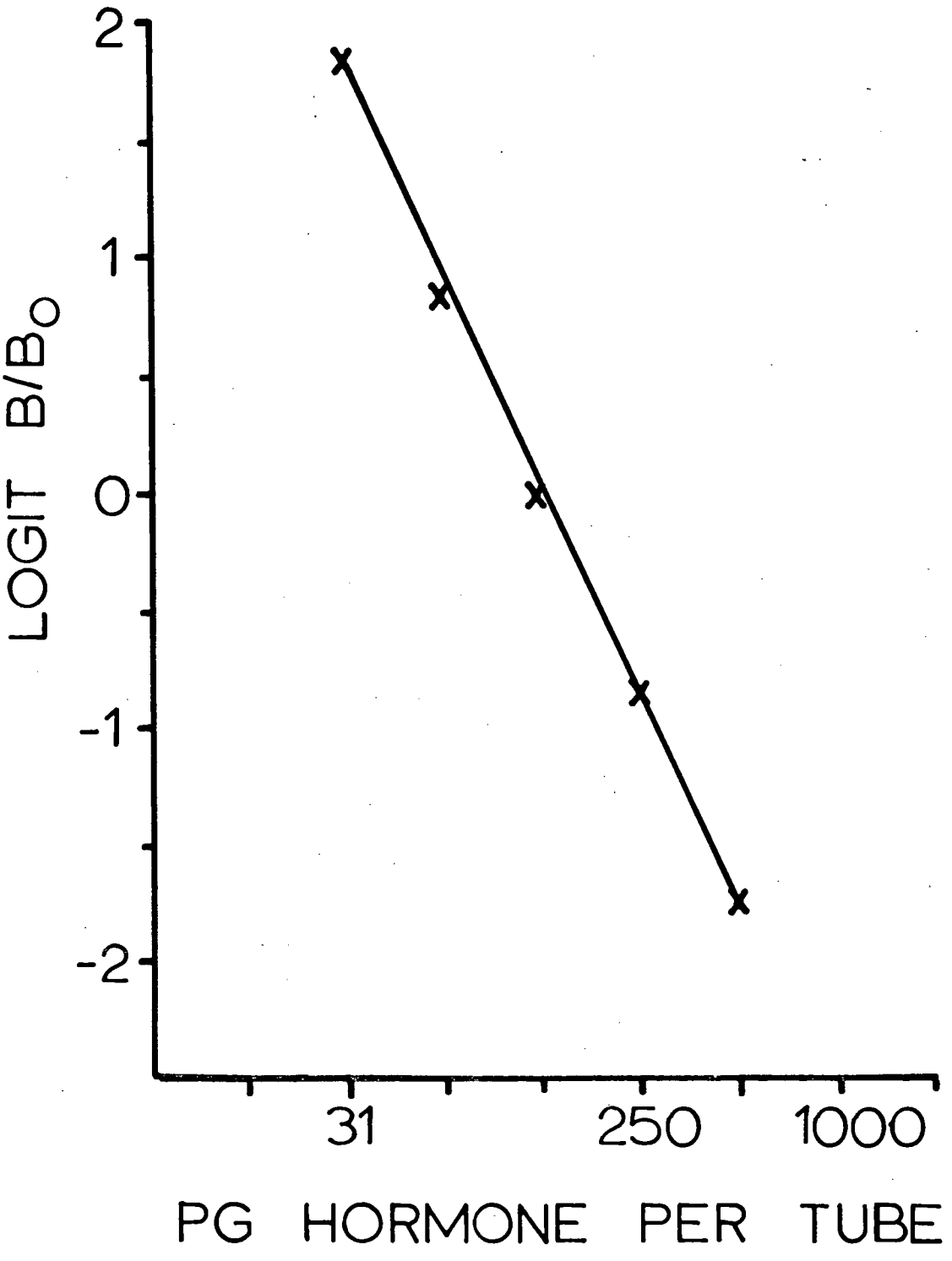


FIGURE 2.15: Standard curve for progesterone.

Oestradiol-17β was extracted from plasma with 1.0ml. of diethyl ether which had been washed with 50% (W/v) ferrous sulphate (BDH) in 5% (W/v) sulphuric acid (BDH) and distilled water (1l. of diethyl ether to 50ml. acidified ferrous sulphate solution and 200ml. of water), and redistilled within 24 hours of use.

The coefficient of variation for recovery between samples within an assay was approximately 9.5%. The range of recoveries was 78.7 - 91.7% in the 5 assays.

Repeated assay of a pool of pregnant female marmoset plasma gave a mean (± S.E.M.) value of 17.25 ± 0.37 ng/ml, with a coefficient of variation of 10.7% (inter-assay variation). Another pool of female marmoset plasma in one assay produced a mean value of 17.46 ± 0.29 ng/ml,, with a coefficient of variation of 5.0% (intra-assay variation; n=9). The limit of detection of the assay (B/Bo = 90%) was 9 ± 2 pg/tube (mean ± S.D.; Chambers and Hearn, in press). With a 100 µl sample, the detection limit was 0.21 ng/ml (assuming an 85% recovery).

A typical standard curve for the oestradiol-17β assay is shown in Figure 2.16.

2.15 Radioimmunoassay for testosterone

Testosterone was measured by the method described by Corker and Davidson (1978). The antiserum to testosterone (EOI, supplied by Dr. S. A. Tillson, Aliza Corporation, Palo Alto, U.S.A.) was raised in a goat immunised with testosterone-3-carboxymethyoxime coupled to bovine serum albumen. Cross reactions of other steroids tested included:- 5α-dihydrotestosterone (23.9%), 11α-dihydroxytestosterone (0.4%), oestradiol-17β (0.2%) and androstenedione (0.1%). This assay also differs from that for progesterone in the following ways:-

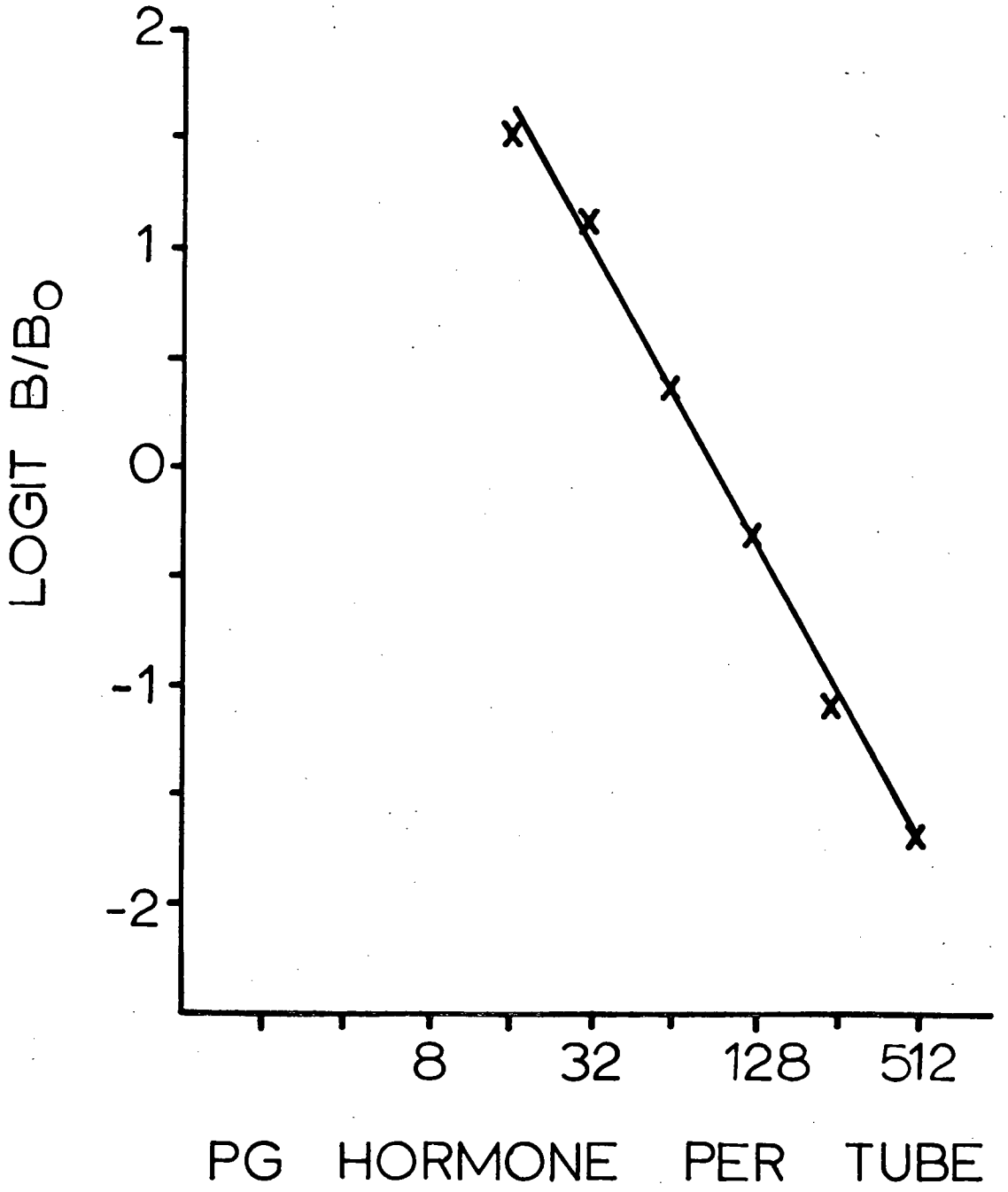


FIGURE 2.16: Standard curve for oestradiol-17β.

Non-radioactive testosterone (Sigma) was dissolved in ethanol to give a concentration of 1 µg/ml., and stored at 4°C. 400 µl were removed, evaporated to dryness, and the residue dissolved in 100ml. of buffer, to give a concentration of 400 pg/100ml. Suitable dilutions of this solution were then prepared to produce concentrations of 400, 200, 100, 50, 25 and 12.5 pg/100 µl buffer. These were used as standards.

Radioactive testosterone (1,2,6,7-^{3}H-testosterone) with a specific activity of 294 µCi/µgm (250µCi in 0.25ml. benzene-ethanol 9:1) was obtained from New England Nuclear and stored at 4°C in ethanol (125 µCi (0.42µg)/ml.). For use in the assay, an aliquot of this solution was dried down under nitrogen and redissolved in buffer to give a solution containing approximately 6000 cpm of 1,2-^{3}H-testosterone per 100 µl. of buffer.

The antiserum was used at an initial dilution of 1:8000 in buffer.

Hexane and diethyl-ether were distilled separately (not more than 24 hours before use) and mixed in a ratio of hexane:ether (4:1). 1.0 ml. of this mixture was used to extract testosterone from plasma.

20 µl of 1,2,6,7-^{3}H-testosterone in ethanol (approx. 1200 cpm) were added to the plasma (duplicate 20 µl aliquots made up to 100ml. with buffer) to test the recovery of testosterone. The coefficient of variation for recovery between samples within an assay ranged from 4.7 - 9.6%. The range of recoveries was 66.7 - 79.3% over the first 20 assays. Thereafter, single recoveries were determined for 25 of the duplicate aliquots of plasma from samples included in the assay. When female samples were assayed, a chromatography stage had to be introduced because the testosterone assay seriously overestimated

plasma testosterone levels in women (Corker and Davidson, 1978). The results in female marmosets have been equivocal so far. The hexane-ether extracts were transferred to alumina columns (Furuyama, Mayes and Nugent, 1970) and the columns washed with 1.6ml. hexane-ether (4:1), followed four times with 1:6ml. of 0.4% ethanol in hexane and the testosterone eluted with 3.2ml. of 1.4% ethanol in hexane. The eluate was evaporated to dryness and dissolved in buffer (as mentioned above).

Repeated assay of a pool of human male plasma gave a mean value ($\pm$ S.E.M.) of 4.64 $\pm$ 0.12 ng/ml and the inter-assay variation, expressed as the coefficient of variation (CoV), was 15.7% (16 assays). Similar treatment of a pool of male marmoset plasma gave a mean value of 15.86 $\pm$ 0.69 ng/ml and an inter-assay variation of 16.9% (CoV: 5 assays). Another pool of human male plasma gave an intra-assay variation of 7.7% with a mean value of 4.62 $\pm$ 0.36 ng/ml (n=18). The sensitivity of the assay (the precision of the measurement of zero (Bo)) was 8pg (Corker and Davidson, 1978). With a 20 µl sample, the detection was 1.1ng/ml. (assuming a 72% recovery).

A typical standard curve for the testosterone assay is shown in Figure 2.17.

2.16 Validation of the steroid assays

Validation of the steroid assays was obtained by comparing the parallelism between standards with serial dilutions of marmoset plasma and by comparing the hormonal concentrations of pooled plasma samples run through the radioimmunoassays with the same samples run through a gas-layered chromatography mass spectrometer (GLC/MS: Kelly, 1971; Kelly and Taylor, 1976).

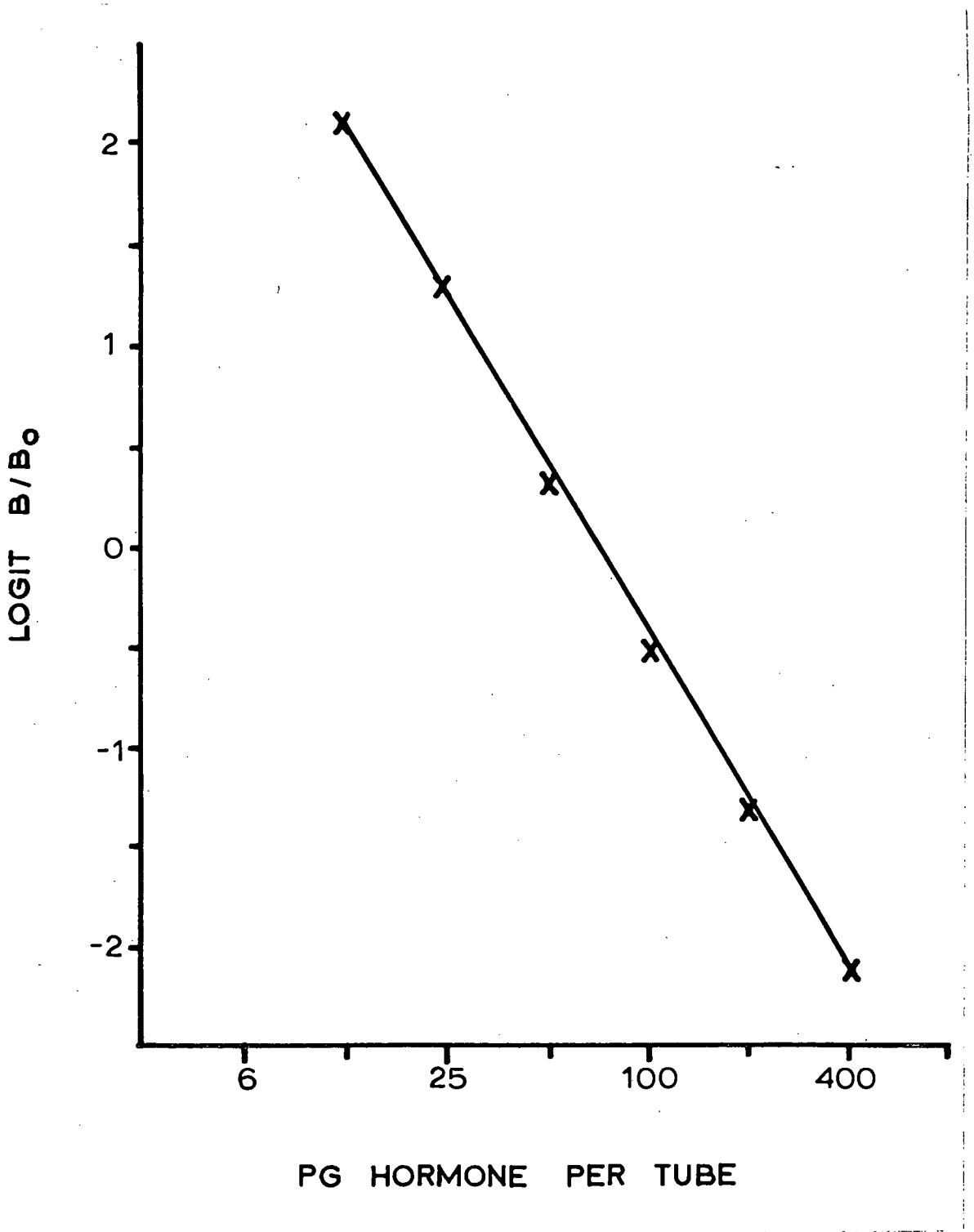


FIGURE 2.17: Standard curve for testosterone.

There was no significant departure from parallelism between standards and dilutions of plasma for progesterone ($p > 0.2$), oestradiol-17 ($p > 0.05$) and testosterone ($p > 0.05$) using a two factor ANOVAR with replication (Chambers and Hearn, in press). Recovery of ligand added to plasma gave regression lines of

$y = 0.985x + 0.451$, $r = 0.999$ for progesterone (1.2-125ng/ml)

$y = 0.978x + 0.526$, $r = 0.999$ for oestradiol-17β (0.3-125ng/ml)

and $y = 1.155x + 0.274$, $r = 0.950$ for testosterone (0.3-9.6ng/ml)

(Chambers and Hearn, in press). Concentrations of plasma oestradiol-17β measured by GLC/MS differed by less than 9% from the same samples measured by radioimmunoassay (Chambers and Hearn, in press), and concentrations of testosterone differed by less than 20% (Corker and Davidson, 1978). Progesterone was not compared because of the instability of the derivative required for GLC/MS (R. W. Kelly, personal communication).

2.17 Radioimmunoassay for luteinizing hormone

Plasma luteinising hormone (LH) was measured using a double antibody radioimmunoassay which was a modification of the assay for rat LH described by Welschen, Osman, Dullaart, De Greef, Uilenbroek and De Jong (1975). The system which was finally adapted to measure LH in marmoset plasma utilised NIAMDD-rat LH I-1 for iodination, NIAMDD - rat LH RP-1 as standard, and anti-ovine LH 610V as antiserum (Hodges, 1977, 1978). The antiserum was raised in a rabbit by immunisation with NIH-LH 517. The assay cross-reacted with NIAMDD rat LH RP-1 by 3%, with bovine FSH (6FSH CH-1-76) and NIAMDD rat FSH I-1 by 0.17% and $< 0.3\%$ respectively, and with all human preparations by less than 0.05% (Hodges, 1977, 1978).

2.17a Reagents

2.17a(i) Buffers

Phosphate buffered saline (PBS; 0.01M, pH 7.8): This buffer was made from stock solutions of 0.4M disodium hydrogen orthophosphate (A) and 0.4M sodium dihydrogen orthophosphate (B). 36 gm. of sodium chloride, 91.6ml. of A and 8.4ml. of B were made up to 4l. with distilled water. 0.04gm. of sodium thiomersalate (Hopkins and Williams, Ltd.) was added as a preservative.

Phosphate buffered saline (PBS; 0.05M, pH 7.8): This buffer was prepared in an identical way to the one above except that the solution was made up to 800ml.

Phosphate buffered saline plus bovine serum albumin (PBS + BSA) : BSA was added to 0.01M. PBS to give a concentration of 1gm. per 100ml. (1% BSA).

"Special Buffer": 3.72 gm. ethylenediaminetetra-acetic acid (EDTA; Sigma) was dissolved in 1l. of 0.01M. PBS. The pH of this solution was adjusted to 7.5 using 3N. sodium hydroxide. Normal rabbit serum (6.7ml. NRS; Wellcome Reagents Ltd.) and 2 gm. BSA were added.

Barbitone buffer (0.12M., pH 8.5): Diethyl barbituric acid (110 gm.) was dissolved in 4.5l. of distilled water, and 19 gm. of sodium hydroxide in 1l. of de-ionised water was added and stirred for 2h. The solution was then made up to 5l. with distilled water and stirred for 24h.

Barbitone buffer plus BSA: 5gm. BSA were dissolved in 100 ml. Barbitone buffer (i.e. 5% BSA).

All buffers were stored at 4°C.

2.17a(ii) Iodination of rat LH

Rat LH preparation NIAMDD-rat LH I-1 was labelled with $Na^{125}I$ (Radio-chemical Centre, Amersham) by a modification of the chloramine-T method of Greenwood, Hunter and Glover (1963). 2 µg of hormone were reacted with 0.5 - 1.0 mCi $Na^{125}I$ and 50 µg chloramine T in a plastic tube (11 x 63 mm., Sarstedt). The chloramine T was made up immediately before use to a concentration of 5mg/ml. in 0.05M. phosphate buffer; 10 µl of this solution was used in the reaction. The reaction was stopped after 30 sec. by adding 125 µg sodium metabisulphide in 0.5ml. 0.05M. PBS. 10 mg. potassium iodide was then added in 0.5ml. 0.12M. barbitone buffer (pH8.6). Labelled LH was isolated from the reaction mixture by absorption chromatography on a small column (1 x 5 cm.) of Whatman CF II grade cellulose (Whatman Pharmaceuticals). The reaction mixture was applied to the column which was then washed with 30 ml. 0.12M. barbitone buffer. The 125_I labelled LH was eluted from the column by passing through 10ml. of barbitone buffer containing 5% BSA, and collecting 1 ml. fractions. The most immunoreactive fractions were pooled and no further purification was necessary.

2.17a(iii) Standards

Standard LH solutions were prepared from 50 µl aliquots (10mg. hormone) stored at -20°C. by diluting to a total volume of 12.5ml. in PBS and 1% BSA buffer. This gave a concentration of 800µg/ml. and standard solutions were prepared as doubling dilutions of this solution. For each assay, standards were aliquoted in duplicate so that tubes contained 160-0.6ng in a volume of 200 µl.

2.17a(iv) Antiserum

Anti-ovine LH 610V was stored at -20°C in 50 µl aliquots at a dilution of 1:10. This was diluted to 1:1000 with 0.01M. PBS and stored at 4°C. For each assay this solution was diluted further in special buffer to give an initial concentration of 1:70,000. This dilution of antiserum bound 20-35% of the labelled tracer (n=4).

2.17b Assay procedure

All samples were assayed in duplicate using plastic 63 x 11mm. tubes (Sarstedt) the protocol of each assay included:-

(1) Total count tubes (TC): ^{125}I-LH (100µl).

(2) Non-specific binding tubes (NSB): special buffer (100 µl);
PBS & 1% BSA (200 µl);
^{125}I-LH (100 µl).

(3) Total bound tubes (TB) : PBS & 1% BSA (200 µl);
antiserum (100 µl);
^{125}I-LH (100 µl.).

(4) Standards: standard LH (200 µl.);
antiserum (100 µl);
^{125}I-LH (100 µl).

(5) Unknowns and quality controls: plasma (50 µl);
PBS & 1% BSA (150 µl);
antiserum (100 µl)
^{125}I-LH (100 µl).

Standards were dispensed in a volume of 200 µl PBS & 1% BSA buffer. Test samples were dispensed in 50 µl. aliquots and made up to 200 µl. with PBS & 1% BSA buffer. Antiserum (100 µl) was added

and the contents of each tube mixed and incubated at 4°C. for 3 days. ^{125}I-labelled LH (approximately 10,000 gm in 100 µl PBS & 1% BSA buffer) was added to all tubes which were mixed and incubated at 4°C. for a further 2 days. Separation of antibody bound and free hormone was achieved by adding 200 µl of donkey anti-rabbit gamma globulin (1:30 v/v in 0.01M. PBS; Burroughs Wellcome, RD17) and incubation was continued at 4°C for 16h. The unbound radioactivity was diluted with 1ml. 0.01M PBS and the tubes centrifuged at 4°C. for 30 min. at 2500 rpm. The supernatants were discarded, the tubes dried with tissue-paper, and the antibody-bound ^{125}I-labelled LH in the precipitate was measured in an automatic gamma spectrometer (Wallac Decam- GT2).

Eppendorf pipettes with disposable tips were used throughout the assay for dispensing the standards and plasma samples. Buffer was added to assay tubes with a semi-automatic dispenser (Lumix), and repettes (Jencons) were used to add iodinated hormone and antiserum to the tubes.

2.17c Precision and sensitivity

The precision of the assay was assessed by repeated assay of a pool of marmoset plasma from gonadectomized males and females. The pool was used to determine inter-assay variation and had a mean value ($\pm$ S.E.M.) of 81.9 $\pm$ 1.2 ng/ml. with a coefficient of variation of 4.3% (4 assays).

The limit of detection of the assay (B/Bo = 90%) ranged from 0.7 - 0.9 ng/tube. Since the dilutions of marmoset plasma and rat standard became non-parallel at above 87% B/Bo (Hodges, 1977, 1978), a working sensitivity of 1ng/tube was adopted. With a 50 µl sample, the detection limit was therefore 20 ng/ml LH RP-1 equivalent.

2.17d Calculations

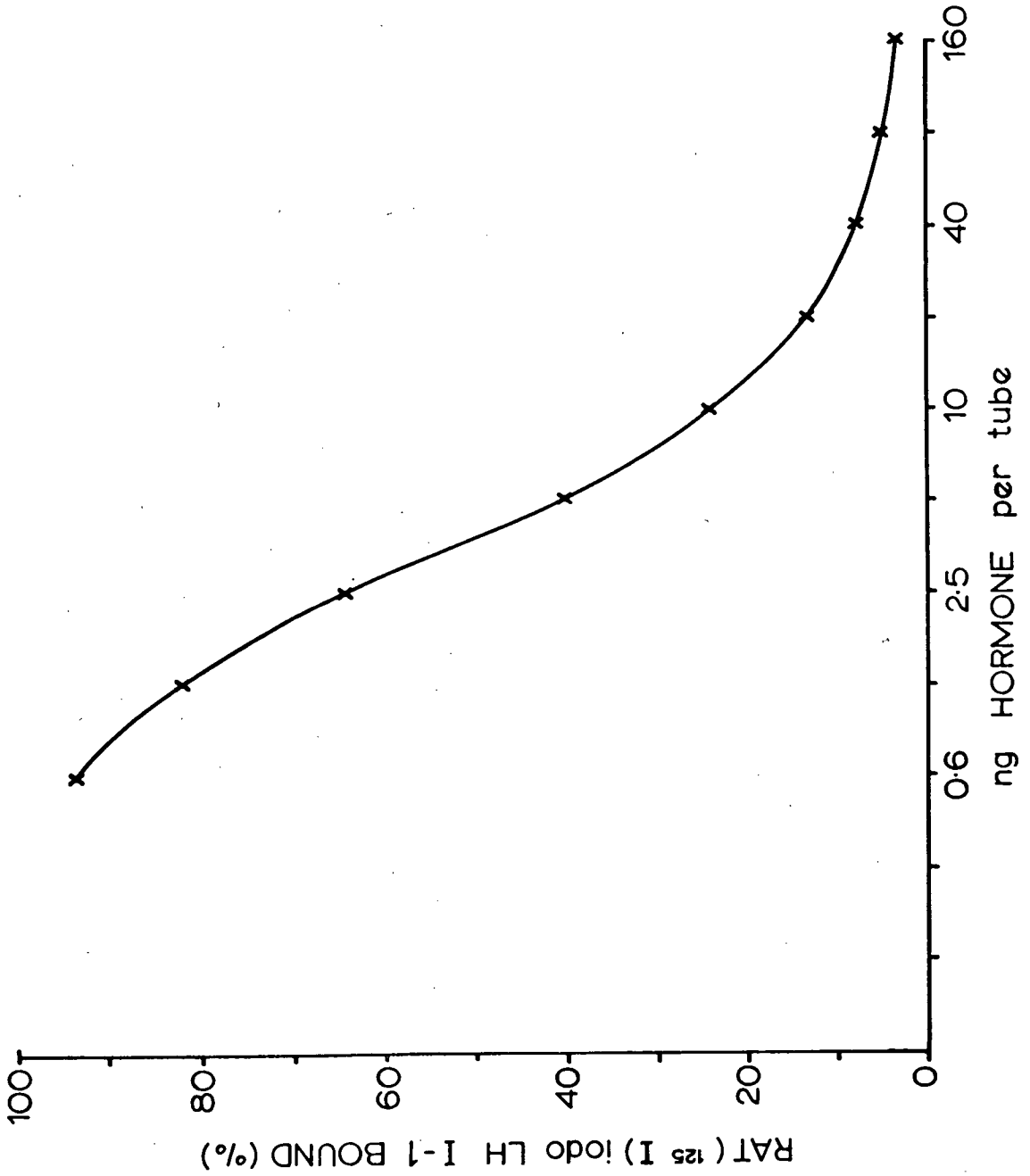
A typically sigmoid standard curve was obtained using a Y-axis of B/Bo and an X-axis of ng. values of the standards on a log scale at a dose interval of two. Figure 2.18 shows a specimen curve. The curve was drawn to pass through the mean value of each dose of standard.

2.17e Validation of the LH assay

Physiological validation of the assay was obtained by measuring LH concentrations after LH-RH administration to intact and LH-RH immunised male marmosets (Hodges, 1977) and the LH response to gonadectomy (Hodges, 1978). In the former case, there was a significant increase in the plasma concentration of LH in the intact males after 30 min. but not in the LH-RH immunised animals. In the latter case there was a significant rise of LH by at least 3 days after gonadectomy (a typical LH castration response) and these high LH levels could be suppressed with implants of steroid hormones (Hodges, 1977, 1978).

Serial dilutions of marmoset plasma and a marmoset crude pituitary extract were sufficiently parallel to the inhibition curve obtained with NIAMDD rat LH RP-1 standard over the range 31-87% B/Bo (Hodges, 1977, 1978) for the purpose of this study. There was no significant departure from parallelism ($p > 0.2$) between serial dilutions of marmoset plasma and the marmoset pituitary extract over the complete range of dilutions (Hodges, 1977). As with the steroid hormone assays, absolute validation of this heterologous assay for marmoset LH must await the purification of marmoset pituitary hormones, but the data obtained (Hodges, 1977, 1978) confirmed that the assay measured marmoset LH.

FIGURE 2.18: Standard curve for LH.



2.18 Radioimmunoassay for prolactin

Plasma prolactin was measured using a double antibody radioimmunoassay fully described by McNeilly and Friesen (1978). The assay was developed for use in the rabbit using ovine prolactin NIH-P-S-6 (26 I.U./mg) as a reference standard and anti-human prolactin 33-9 raised in a guinea pig as antiserum. The assay cross-reacted with prolactin preparations from the sheep, cow and pig by 80% and more, with dog (Schering) by 70%, with rabbit (McNeilly 76-1-MC) by 17% and with rat NIAMDD P-I-1 by < 0.1%. Growth hormone, placental lactogen, LH and FSH preparations from many species all cross-reacted by less than 0.1% (McNeilly and Friesen, 1978).

2.18a Preparation of reagents

0.01M. PBS, 0.05M. PBS and PBS + 1% BSA: identical to the recipes in the LH assay (section 2.16a (i)).

0.1% and 1%-Tris-HCl buffer (0.025M; pH 7.5): 3.025gm./l. of 2-amino-2-(hydroxymethyl)propane-1,3-diol, (tris) (Tris(hydroxymethyl) methylamine; BDH) and 1gm./l. (0.1%) or 10 gm./l. (1.0%) of BSA were dissolved in distilled water and the pH adjusted to 7.5 with hydrochloric acid (HCl).

Physiological saline (0.9% ($^{W}/v$) NaCl; pH 7.2): 9 gm. of sodium chloride (NaCl) were added to every litre of distilled water.

Iodination of ovine prolactin

Ovine prolactin (o PRL) NIH-P-S-6 was labelled with $Na^{125}I$ (Radiochemicals Centre, Amersham) by the lactoperoxidase method (Thorrel and Johansson, 1971) at room temperature, as described previously (Shin, Kelly and Friesen, 1973). 5 µg. of the hormone were reacted with approximately 1mCi $Na^{125}I$, 5 pg of lactoperoxidase

and 10 µl of hydrogen peroxidase solution (50 µl in 750 ml. distilled water) in a plastic tube (11 x 63 mm., Sarstedt). The lactoperoxidase was made up immediately before use to a concentration of 0.5 ng/ml. in 0.05M. PBS; 10 µl of this solution was used in the reaction. The labelled hormone (ovine (^{125}I) iodo-PRL) was separated from aggregate hormone and ^{125}I by gel filtration on Sephadex G-100 (2 x 50 mm.) with Tris-HCl buffer. The ^{125} labelled prolactin was stored at -20°C. until used for assay (when diluted to initial dilution of 1:200 with PBS & 1% BSA). The specific activity was assessed by trichloroacetic acid precipitation and ranged between 84-165 µCi/µg. (McNeilly and Friesen, 1978).

Standards

Standard prolactin solutions were prepared from 20 µg of the hormone stored at -20°C. in 1ml. of 0.1% BSA-Tris-HCl buffer. 100µl of this solution was removed and made up to 10 ml. with 0.01M PBS to give a concentration of 200 ng/ml. Standard solutions were prepared as doubling dilutions of this solution to give a range of 200-0.4ng/ml. For each assay, standards were aliquoted in duplicate and at the same volume as the plasma samples (50 µl).

Antiserum

Anti-human prolactin 33-9 was stored at -20°C. in 1 ml. aliquots at a dilution of 1:100. For each assay this was diluted to an initial concentration of 1:6000 with PBS & 1% BSA. This dilution of antiserum bound between 30 and 50% of the labelled tracer (McNeilly and Friesen, 1978).

2.18b Assay procedure

All samples were assayed in duplicate using plastic 63 x 11 mm. tubes (Sarstedt). The protocol of each assay included:

1. Total count tubes (TC): ^{125}I - PRL (100µl)
2. Non-specific binding tubes (NSB): PBS & 1% BSA (450µl)
   ^{125}I - PRL (100µl)
3. Total bound tubes (TB): PBS & 1% BSA (350µl)
   antiserum (100µl)
   ^{125}I - PRL (100µl)
4. Standards: standard prolactin (50µl)
   PBS & 1% BSA (300µl)
   antiserum (100µl)
   ^{125}I - PRL (100µl)
5. Unknowns and quality controls: plasma (50 µl)
   PBS & 1% BSA (300µl)
   antiserum (100µl)
   ^{125}I - PRL (100µl)

Standards and unknowns were dispensed in a volume of 50 µl and made up to 350µl with PBS + 1% BSA buffer. Antiserum (100µl) was added and the contents of each tube mixed and incubated for 24h. ^{125}I-labelled prolactin (approximately 20,000 cpm in 100µl (PBS & 1% BSA) was added to all tubes which were mixed and incubated at 4°C. for a further 3 days. Separation of antibody bound and free hormone was achieved by adding 100µl of normal guinea pig precipitating serum (1:600 v/v in 0.01M. PBS) and then 100 µl of donkey anti-guinea pig gamma globulin (1:15 v/v in 0.01M. PBS; Burroughs Wellcome). Incubation was continued at 4°C. for another 16h. The unbound radioactivity was then diluted with 1.0ml. of physiological saline and the tubes

centrifuged at 4°C. for 30 min. at 2500 rpm. The supernatants were discarded, the tubes dried with tissue paper, and the antibody-bound ^{125}I-labelled prolactin in the precipitate was measured in an automatic gamma spectrometer (Model 1270, Rackgamma; Wallac Decam).

A fully automatic pipette (Micromedic Systems Inc.) was used throughout the assay for dispensing the standards and plasma samples. Saline was added to assay tubes with a semi-automatic dispenser (Lumix) and repettes (Jencons) were used to add iodinated hormone, antisera and normal guinea pig precipitating serum to the tubes.

2.18c Precision of the assay

The inter- and intra-assay variation has been previously assessed by McNeilly and Friesen (1978) at 10.4-12.6% and 6.4-7.6%, respectively. In this study, the repeated assay of a pool of ovine plasma in one assay gave a mean value ($\pm$ S.E.M.) of 43.9 $\pm$ 1.1 ng/ml. with a coefficient of variation of 7.5%.

2.18d Calculations

The procedure was identical to that in the LH assay (section 2.16d) and a typical sigmoid standard curve is shown in Figure 2.19.

2.18e Validation of the prolactin assay

Physiological validation of the assay was obtained by measuring prolactin concentrations after -

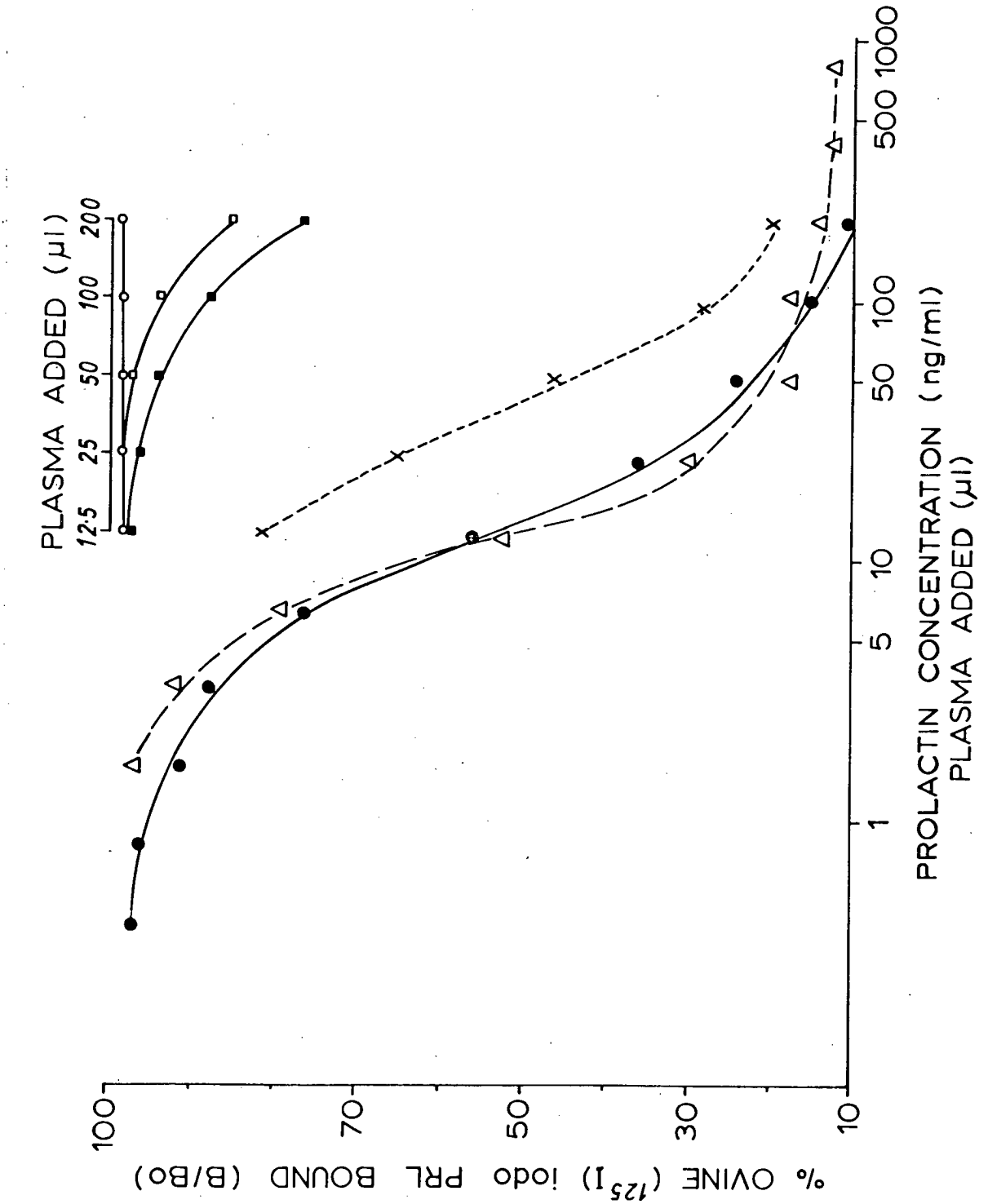
(a) TRH administration,

(b) metoclopramide administration, and

(c) bromocriptine (CB154) administration,

to five cycling female marmosets (Figure 2.20). TRH and metoclopramide were known to stimulate prolactin concentration and bromocriptine

FIGURE 2.19: Standard curve for prolactin (● ; rat NIH-S-6 PRL) and dilution curves of: (a) a marmoset pituitary extract (▲); (b) female marmoset plasma (x; after metaclopramide stimulation); (c) female marmoset plasma (O; after bromocriptine administration); (d) male marmoset plasma (□); (e) female marmoset plasma (■).



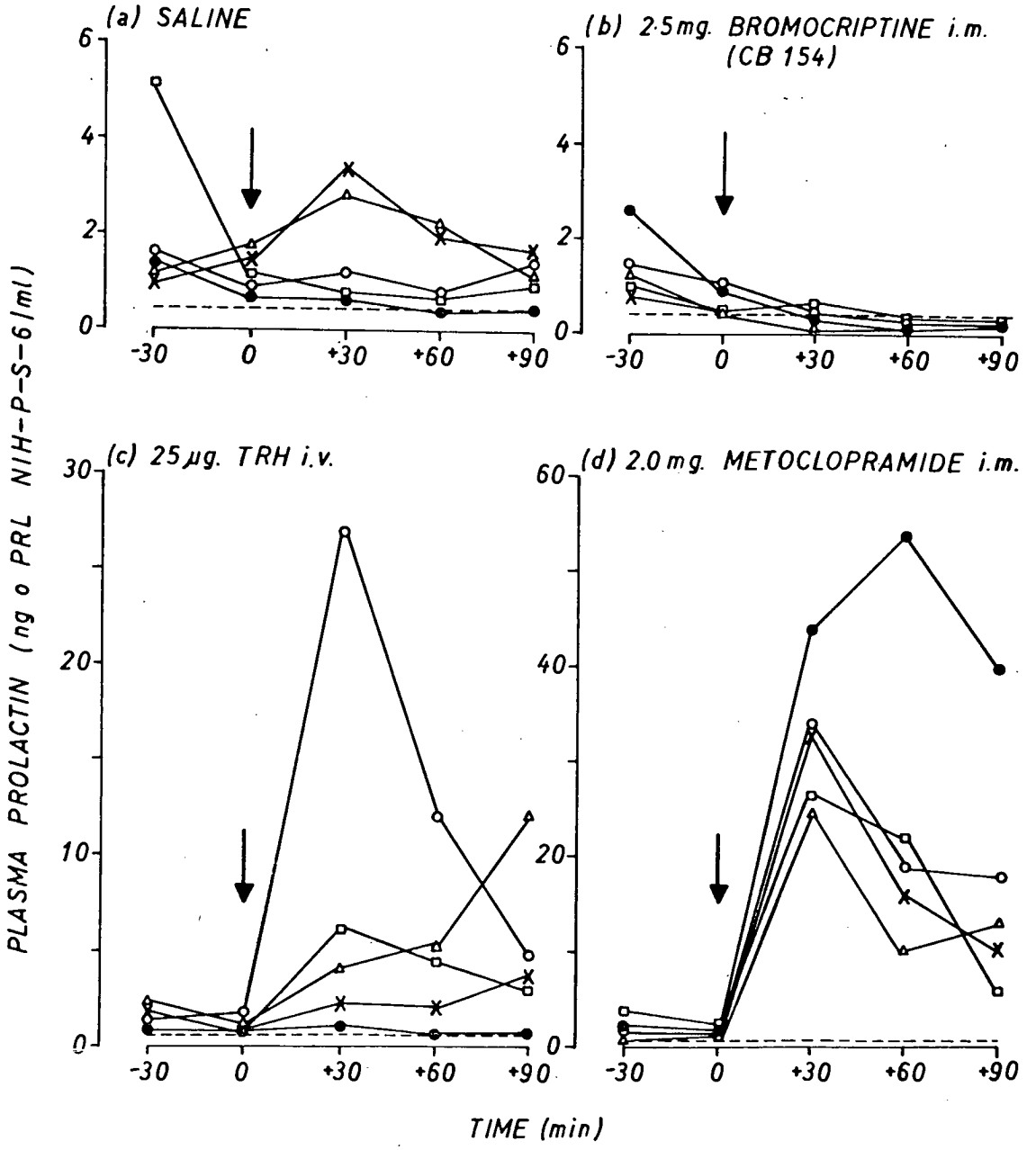


FIGURE 2.20: Effects of (a) physiological saline, (b) bromocriptine, (c) TRH and (d) metaclopramide on the concentrations of plasma prolactin in cycling female marmosets.

● = Female 1
O = Female 2
Δ = Female 3
□ = Female 4
X = Female 5.

(a) and (b) by courtesy of McNeilly, Abbott, Hearn and Lunn (unpublished work).

depresses it (McNeilly and Friesen, 1978). In female marmosets TRH stimulated prolactin concentrations in three and metoclopramide in all five. This result was much as expected from the responses in female rabbits (McNeilly and Friesen, 1978). Bromocriptine administration depressed prolactin levels below the theoretical detection limit of the assay (0.4ng/ml. standard) indicating that there were no plasma factors interfering with the assay and giving falsely elevated results (Figure 2.20). An injection of saline followed by repeated venepuncture produced a relatively negligible prolactin response (<0.4 ng/ml), indicating that stress from the bleeding schedule was not a significant factor in elevating circulating prolactin concentrations.

For the purposes of this study, the serial dilution of marmoset crude pituitary extract was sufficiently parallel to the inhibition curve obtained with the ovine standard over the range 10-92% B/Bo ($p > 0.05$, ANOVAR: Figure 2.19), even though the slope of the pituitary extract curve was steeper than that of the ovine standard. Serial dilutions of plasma from cycling female marmosets stimulated with metoclopramide, and intact male and female marmosets were parallel to the ovine standard over their complete range of dilutions ($p > 0.05$). Serial dilutions of plasma from metaclopramide stimulated female marmosets and plasma dilutions from unstimulated male and female marmosets were parallel to the serial dilutions of marmoset pituitary extract ($p > 0.05$). No inhibition of binding occurred with plasma from bromocriptine-treated animals, again eliminating any possible plasma interference (Figure 2.19). As with other assays, while purified marmoset prolactin is not available to provide absolute validation, this heterologous assay system responds sufficiently well to permit the use of the ovine preparations as standard equivalents to marmoset prolactin.

2.18 Assay kit for cortisol

Plasma cortisol was measured using a competitive protein binding kit ('Cortipac'; The Radiochemical Centre, Amersham). The assay was developed for use in the human using cortisol contained in lyophilized human reference serum as a standard and adsorbent granules and buffered transcortin solution as the competitive proteins. The assay cross-reacted completely with cortisone and 11-dexoycortisol (100%), and an "intermediate degree" of cross-reaction was observed with progesterone, prednisone, predinsdone, corticosterone and a number of other related corticosteroids (Technical Bulletin 75/10; The Radiochemicals Centre). In most cases these cross-reactions were deemed unimportant, in view of the much higher level of circulating cortisol except, for example, in late pregnancy, where the interference of high levels of progesterone could not be ignored.

2.19a Contents of each kit

Each Cortipac kit contained 25 test vials. Each test vial contained, within narrow limits, the same weight of adsorbent granules and the same volume of buffered transcortin-cortisol (^{75}Se) solution (not more than 0.2 μCi ^{75}Se per vial).

Four other vials contained lyophilizedhuman reference serum holding 10, 32.5, 84.5 and 239μg. cortisol respectively. The sera were previously calibrated against absolute standards (Radiochemical Centre).

25 vials were supplied for the heat denaturation stage. All the vials were stored at $4^{o}C$. until used.

2.19b Reconstitution of the standards

500 µl. of distilled water was added to each vial of standard and the freeze-dried serum left to dissolve at room temperature for 10 min. The contents of each vial were then mixed thoroughly and either used immediately or stored at -20°C. for up to 2 weeks. This dilution provided reference standards of 2.0, 6.5, 16.9 and 47.8 µg/100ml.

2.19c Assay procedure

Marmoset plasma was first diluted to 1:20 (v/v) with distilled water, to match the range of cortisol concentration covered by the standards. This procedure followed the serial dilution of two female marmoset samples which provided counts at approximately the middle of the standard at the above dilution (37,587 and 30,428 counts/100 sec., respectively; see Figure 2.21).

Duplicate aliquots of 100 µl of standards or diluted unknowns were then dispensed into the glass denaturation vials. A further 200µl of distilled water was added to each tube and the contents were thoroughly mixed. The rack of tubes was placed in a covered water bath at 70°C. for 10 min. in order to denature the endogenous cortisol binding protein (transcortin) and release the cortisol. The tubes were allowed to cool to room temperature and 200 µl of the denatured sample was pipetted into an assay vial. Each vial was capped and the contents mixed continuously at room temperature in a multivortex mechanical shaker (Baird and Tatlock) for 45 min. (the minimum recommended time was 30 min.). Cortisol from the serum or plasma samples and selenium-75 labelled cortisol competed for binding sites on the transcortin. The free fraction was simultaneously distributed

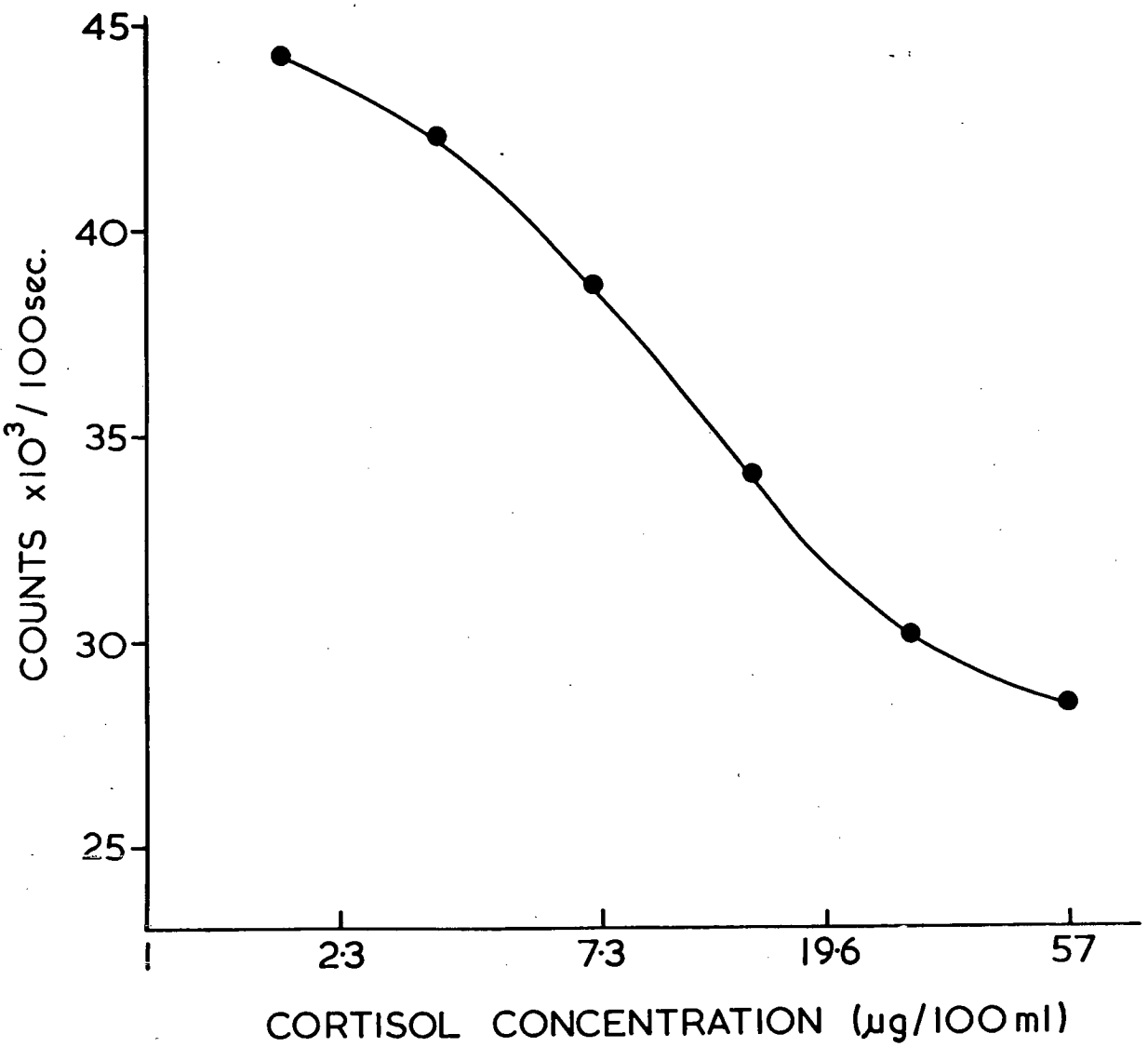


FIGURE 2.21: Cortisol: (a) a standard curve of lyophilized human serum cortisol.

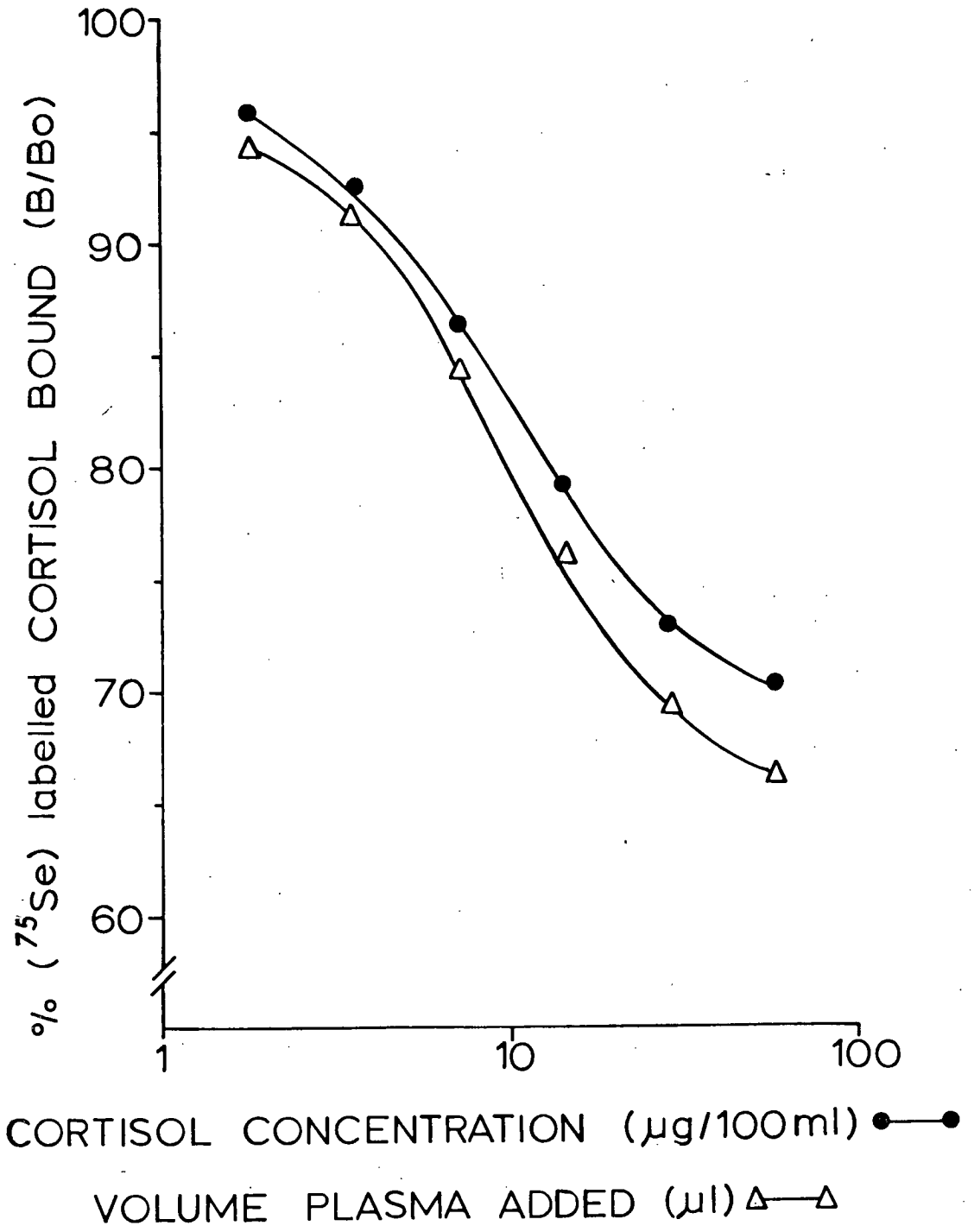


FIGURE 2.21: Cortisol: (b) a standard curve of lyophilized human serum cortisol (●) and a dilution curve of marmoset plasma cortisol (Δ).

between the granules and the supernatant. At equilibrium (more than 30 min. of mixing) the granules were allowed to settle and 500 µl of the supernatant was taken for counting on an automatic gamma spectrometer (Wallac Decem-GT2), calibrated for counting selerium-75.

2.19d Precision and sensitivity

Two pools of female marmoset plasma (diluted to 1:20) were used to determine inter- and intra-assay variation. One pool was repeatedly assayed in duplicate in 5 assays and gave a mean value ($\pm$ S.E.M.) of 100.8 $\pm$ 3.5 µg/100ml with a coefficient of variation between assays of 10.4%. The other pool was repeatedly assayed in one assay and gave a value of 99.6 $\pm$ 3.5 µg/100ml. with a coefficient of variation within an assay of 7.8% (n=8).

Only values of samples within the range of standards were calculated.

2.19e Calculations

A standard curve was constructed on the results sheet provided (e.g. Figure 2.21). The counts for standards were plotted on the Y-axis against cortisol concentration on the logarithmic X-axis. The values of the unknowns were read off from this best fit line. The recommended 50,000 counts/100 sec. for a 2.5 µg standard (Radiochemical Centre) was similar to the 40-45,000 counts/100sec. obtained for the 2.0 µg standard in these 6 assays.

2.19f Validation of the assay

Serial dilutions of marmoset plasma was sufficiently parallel to the inhibition curve obtained with human cortisol standard over the complete range of the letter (Figure 2.21; $p > 0.05$, ANOVAR).

The cortipac assay kit has been validated in many clinical situations (Radiochemical Centre) and has shown good correlations with fluorometric (Gore and Lester, 1975) and radioimmunoassay methods (Baum, Tudor and Landon, 1974).

2.20 Surgery

Surgery was performed under aseptic conditions. All animals for surgery were newborn or pre-prepubertal, and were anaesthetised with an intra-muscular injection of 0.05-0.3ml. "Saffan" (18mg/kg. of body weight; Alphaxalone 0.9% $^{w}/_{v}$, Alphadolone acetate 0.3% $^{w}/_{v}$; Glaxo Laboratories Ltd.) except those receiving testosterone implants. This dose of general anaesthetic was sufficient to maintain animals under controlled, deep anaesthesia for a period of about 1 hour. When implants were inserted or removed only a local anaesthetic was given subcutaneously (0.2ml. "Lidothesin", 1% (10mg/ml.) plain: Lignocaine hydrochloride B.P.; Pharmaceutical Manufacturing Co. Ltd.). The animals receiving implants were less than 60 days old and easily restrained with masking tape to an operating board. All the animals received an intra-muscular injection of 0.2ml. penidural (Fortified Injection Veterinary - John Wyeth and Brother). When either anaesthetic had taken effect, the animals were shaved in the appropriate region, the skin disinfected with Hibiscrub (I.C.I. Pharmaceuticals Ltd.) and the surrounding area covered with surgical dressing.

2.21 Surgical procedures

In the course of this study the following surgical procedures were performed:-

1. Bilateral orchidectomy.
2. Bilateral ovariectomy.

3. Insertion and removal of testosterone implants.

4. Sham operations.

1. Bilateral orchidectomy

Two 4-6mm. incisions were made in the skin on the left and right side of the inguinal region with a scalpel blade (all the males were newly-born with undescended, but palpable testes). Each testis and epididymis were gently squeezed out through the incision using forceps. The spermatic cord of each testis was ligated in two places (about 2-3mm. apart) about 3-5mm. along its length from the epididymis, and the testis and epididymis removed after cutting between the ligatures. A thin film of penicillin powder was applied to both wounds and the incisions were closed with two stitches. 3/0 Chromic sutures with a 16mm. cutting needle (Ethicon Ltd.) were used.

2. Bilateral ovariectomy

A 5-10mm. vertical incision was made through the skin in the ventral region of the abdomen, immediately to the right of the midline. A similar incision was made through the muscle layer, taking care to leave the peritoneum intact. The abdominal cavity was then exposed by cutting through the peritoneum using scissors. On each side, the top part of the Fallopian tube and the ovarian pedicle, artery and vein were ligated in two places (2-3mm. apart) with 3/0 Chromic suture. Each ovary was removed after cutting through the attachments between the ligatures with scissors. The incisions in the peritoneum and muscle layer were closed with a continuous stitch (4/0 Chromic suture) and the tissue carefully aligned. After the application of penicillin powder the incision in the skin was closed with a continuous subcuticular purse stitch and then with two or three loose surface stitches.

3. Insertion and removal of testosterone implants

A 3-5mm. incision was made through the skin and connective tissue in the ventrolateral region of the abdomen. The incision was held open with forceps while the 25mg. implant (6-8mm. long and 2mm. in diameter; Organon Ltd.) was introduced with a second pair of forceps to lie at least 10mm. below the incision. Penicillin powder was applied and the incision closed with two stitches.

When the implants were removed, a similar incision was made under local anaesthetic. The implant was removed with forceps and the incision was closed with two stitches.

4. Sham operations

Animals were sham-operated for control purposes in each of the above experimental procedures. In every case an identical incision was made in the control as in the experimental animals. However, no further surgery was performed on the control animals and the wound was stitched up.

CHAPTER 3 PATTERNS OF GROWTH AND PHYSICAL DEVELOPMENT

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3.1 Introduction

Relatively little is known about growth in the subhuman primates in comparison to man (Tanner, 1962; Marshall, 1974). Measurements of growth of marmosets in captivity (Wettstein, 1963) and in the wild (R.H. Gilmore cited by Hershkovitz, 1977) have been reported, but their sample sizes of 7 and 8 respectively (young and adults combined) provide little information. Epple (1970) provided the first sample of body weight data in marmosets of up to $2\frac{1}{4}$ years old, but each animal was only measured once. Recently, Kingston (1975) and Turton et al (1978) have produced comprehensive growth curves for marmoset body weight, but few other measurements have been reported (Hershkovitz, 1977). Hence, marmoset monkeys in our colony were measured from birth until maturity for body weight, knee-to-heel length, and genital and mammary gland size. Normal patterns of growth were established to enable comparison of the physical development of this monogamous monkey with that of polygamous primates and man.

3.2 Procedure

3.2a Body weight and knee-to-heel length

Measurements were taken of body weight and knee-to-heel length in 20 male and 19 female marmosets at 10-day intervals from birth to 200 days of age, and then at 50-day intervals until 600 days. Body weight was recorded from an electronic balance accurate to 0.5gm, and knee-to-heel length was measured with calipers accurate to 0.5mm. Adult size was considered to have been attained when there was no significant difference ($p > 0.05$; Student's t-test) between the young colony-bred animals and the adults caught in the wild

(n=40; 20 males and 20 females). The body weights of females were excluded from the time they became pregnant, and all these measurements were compiled from the colony records.

Weight gain and the rate of knee-to-heel length growth were calculated at 50-day intervals from birth to 600 days of age in 20 males and 20 females. Measurements before and after the inflexion point in the velocity curve were compared using a paired t-test.

3.2b Pudendal pad and mammary glands

The width of the pudendal pad was measured with calipers in 53 young females and 20 adult females. The pudendal pad, a secretory area (Epple, 1973; Hampton and Hampton, 1975), surrounds the vulva in females and the penis and scrotum in males, and is used in marking objects or cage mates (Epple, 1973) or during aggressive display (Stevenson and Poole, 1976). The mammary glands were also measured with calipers and examined for any signs of growth.

3.2c Testis volume

The length and width of the right testis were measured with calipers in 55 young and in 20 adult males. Testes were measured in the scrotum, and their volume was calculated by using the formula for the volume of an oblate spheroid ($V = \frac{1}{6} \pi W^2 L$, where V = volume, W = width, and L = length).

3.3 Results

3.3a Body weight and knee-to-heel length

Figure 3.1 shows growth curves for body weight and knee-to-heel length for marmoset monkeys between birth and 600 days of age,

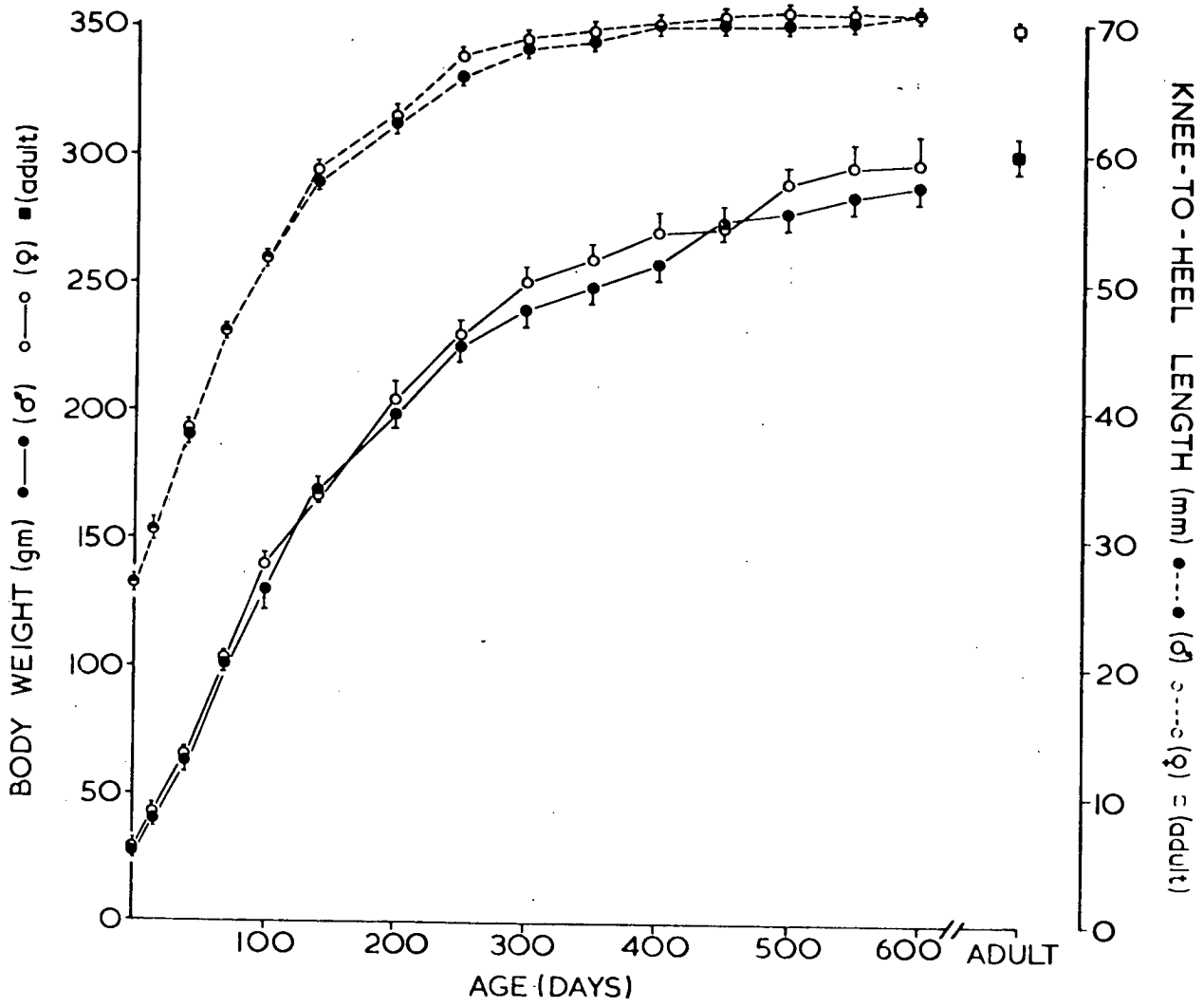


FIGURE 3.1: Growth of male (●) and female (o) marmosets in captivity, determined from measurements of body weight (—) and knee-to-heel length (---). Values are mean ± s.e.m., and at end point 200 days: maximum age range = 2 days, no. of males = 17-20, no. of females = 15-19; at each point 200 days: maximum age range = 18 days, no. of males = 10-20, no. of females = 9-19. The mean ± s.e.m. body weight (■) and knee-to-heel length (□) of 20 adult males and 20 adult females are also shown.

by which time adult size had been attained. There was no significant difference between males and females either in their adult weight or knee-to-heel size ($p > 0.05$; Student's t-test), or in their rates of growth (because of the large overlap of the standard errors at every age). Marmosets reached the adult body weight of 300.0 $\pm$ 6.6gm. (mean $\pm$ s.e.m., n=40) at 500-550 days, and adult knee-to-heel length of 69.7 $\pm$ 0.6 mm. at 300-350 days. The variation in knee-to-heel length between animals of the same age was low between birth and 300 days.

A detailed analysis of weight gain revealed that the maximum weight gain occurred just after birth, between 50 and 100 days, while the maximum for knee-to-heel length occurred at the time of parturition (Fig. 3.2). The rate of body weight gain did not fall in either sex until 100-150 days of age, and then it declined steadily until puberty (see Chapter 4), when there was another increase in both sexes. The increase in weight gain was significant in males, but was only significantly different from the immediately preceding measurement (Table 3.1). There was no corresponding increase in the rate of knee-to-heel length growth.

3.3b Pudendal pad and mammary glands

At birth, the pudendal pad was dark brown in both sexes and between 45 and 60 days of age it became a light pink (Fig. 3.3 and 3.4). There was little difference between the sexes in the appearance of the pudendal pad and external genitalia until the males' testes had reached an appreciable size (between 250 and 350 days of age). Figure 3.5 shows the growth of the pudendal pad in female marmosets:

FIGURE 3.2: Rates of weight gain (——) and knee-to-heel growth (---) in male (●) and female (o) marmosets over 50-day intervals. Values are mean ± s.e.m., and at each point the sources of the data are identical to Figure 3.1.

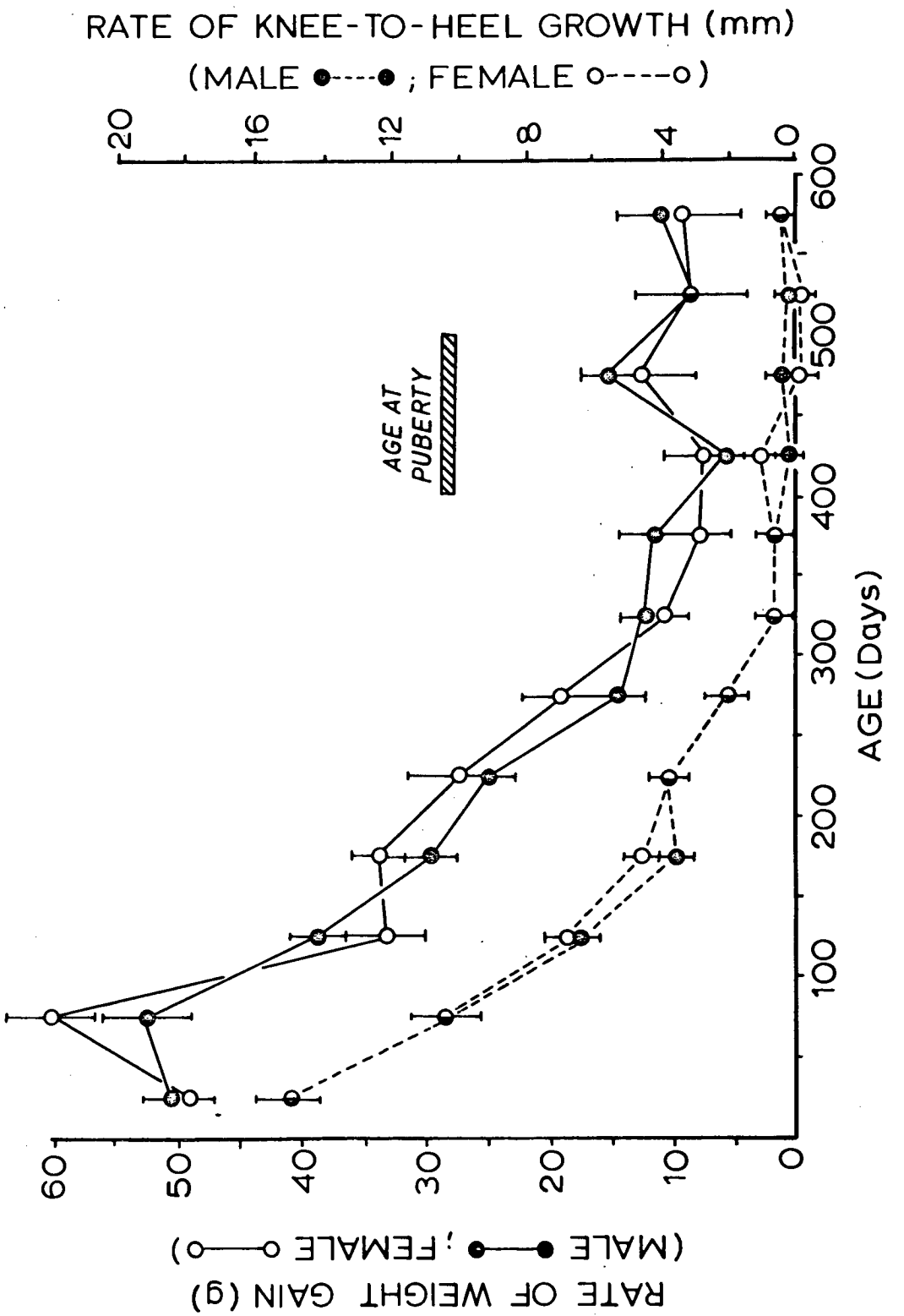


Table 3.1: Rates of body weight gain in (a) male and (b) female marmosets between 350 and 600 days of age.

(a) Males

AGE (DAYS)	n	RATE OF WEIGHT GAIN (gm./50 days)	
350 - 400	16	11.6 ± 2.8†	
400 - 450	16	5.8 ± 3.1	**
450 - 500	18	15.2 ± 2.3	**
500 - 550	17	8.6 ± 3.3	
550 - 600	14	10.9 ± 2.6	

(b) Females

AGE (DAYS)	n	RATE OF WEIGHT GAIN (gm./50 days)
350 - 400	20	7.8 ± 2.9
400 - 450	19	7.5 ± 3.1
450 - 500	16	12.6 ± 4.3
500 - 550	13	8.5 ± 4.4
550 - 600	10	9.2 ± 8.6

† Mean (± s.e.)

** $p < 0.01$ (paired t-test).

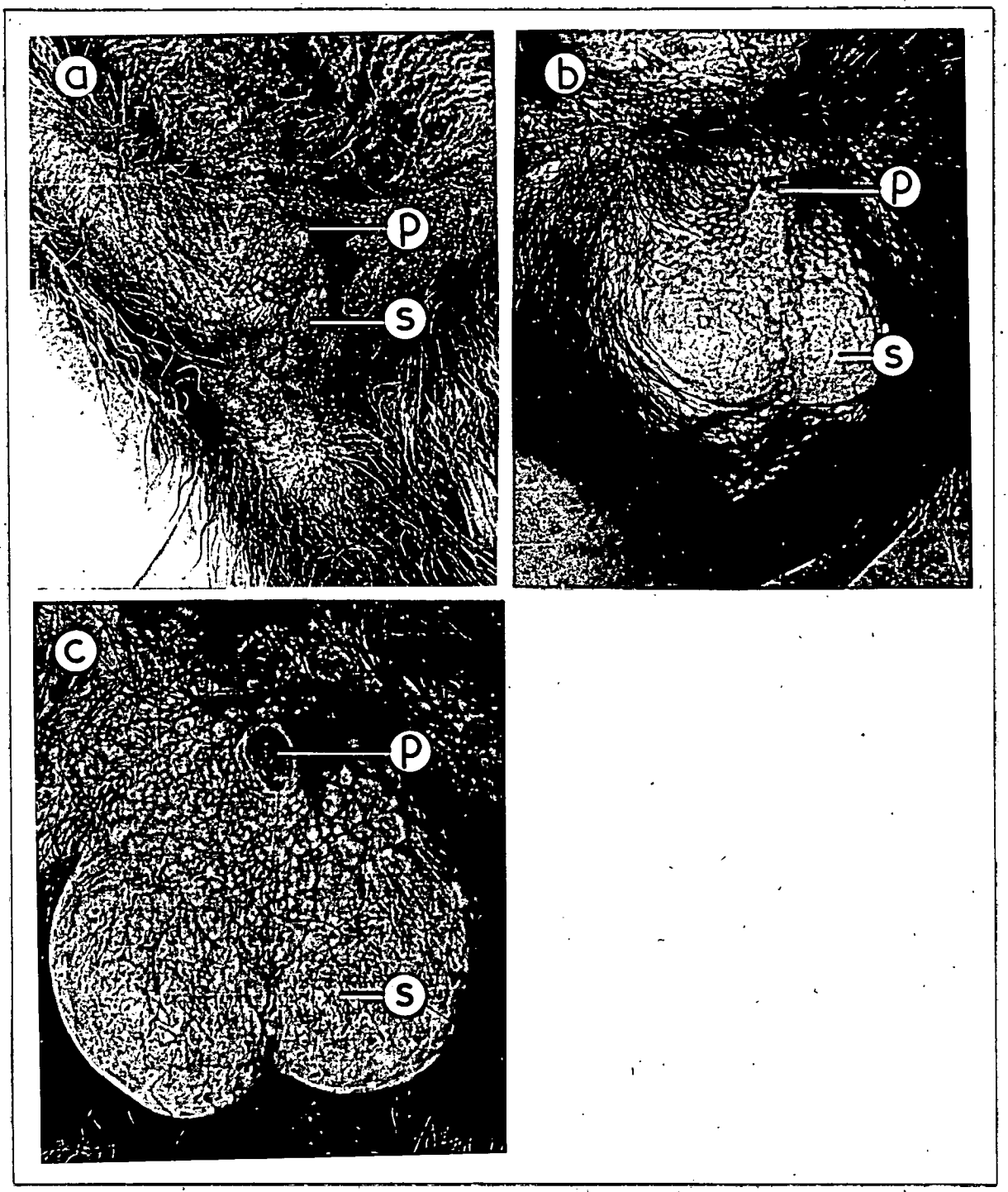


FIGURE 3.3: The external genitalia of male marmosets when (a) 20 days old, (b) 250 days old, and (c) adult. The perineal area is shown and the light-coloured nodular pudendal pad only highlights the genitalia in the pubertal and adult animals. In (a), the poorly developed penis (p) is obscured by the prepuce and the testes have not yet descended into the scrotum (s). In (b), the developing penis (p) is now visible through the prepuce and the growing testes are normally retained in the scrotum (s). In (c), the mature male has a well-developed penis (p) and the testes fill the scrotum (s). (a) and (b) are twice, and (c) is three times the normal size.

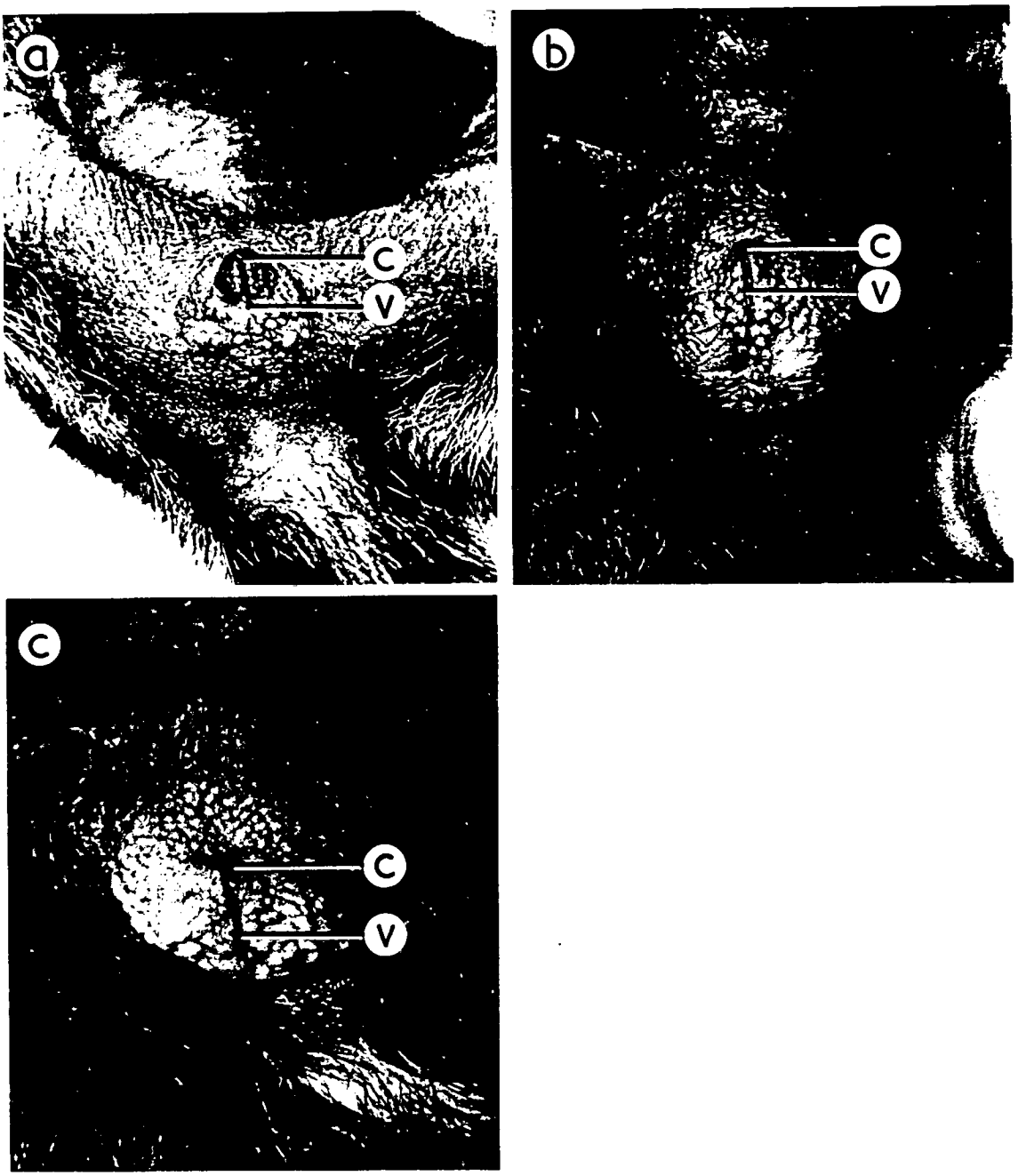
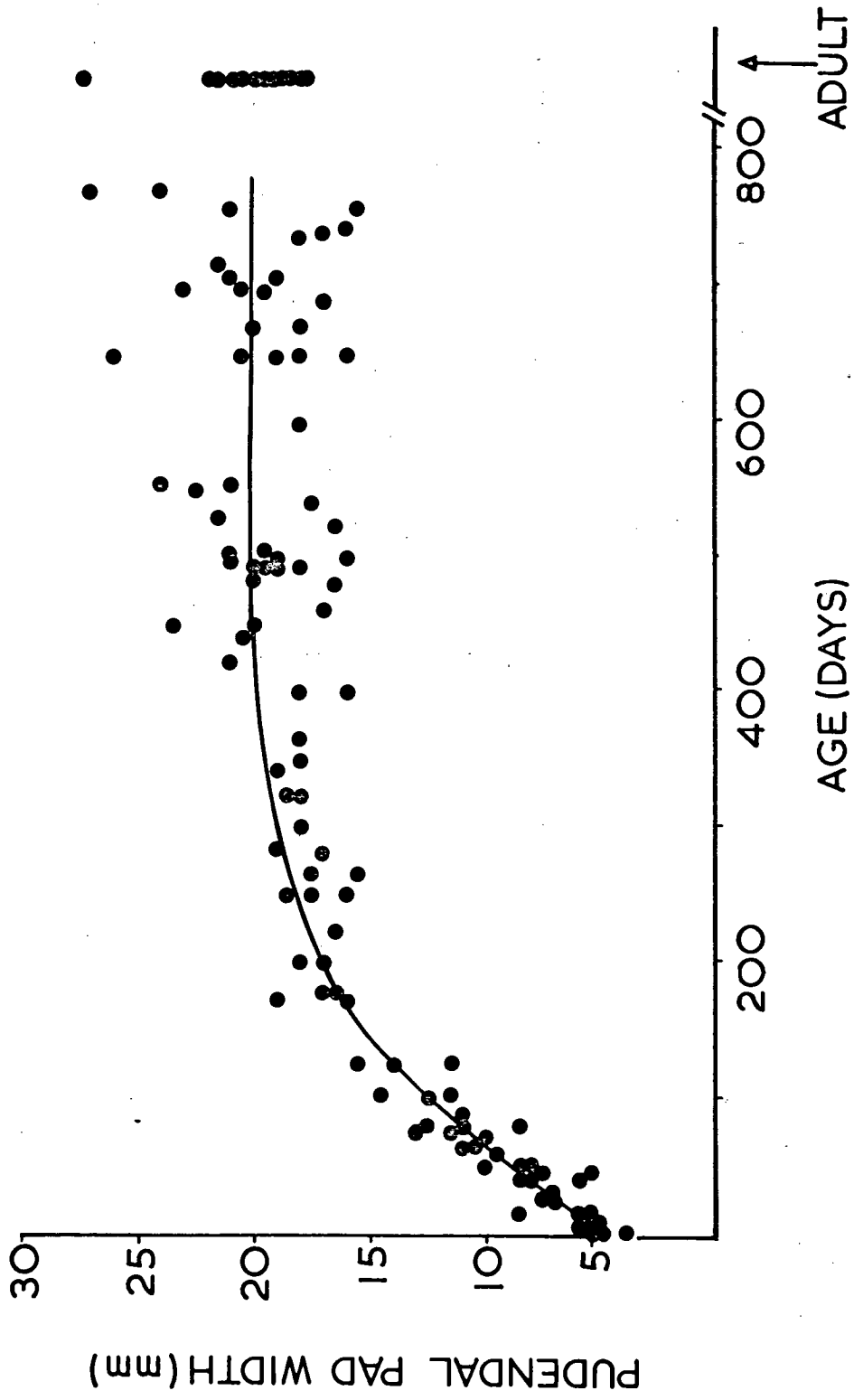


FIGURE 3.4: The external genitalia of female marmosets when (a) 20 days old, (b) 250 days old, and (c) adult. The perineal area is shown and the light-coloured nodular pudendal pad only highlights the genitalia in the pubertal and adult animals. In (a), the clitoris (c) is obscured completely by the labia and the vaginal opening (v) is not obvious. In (b), the clitoris is still slightly obscured by the labia and the vaginal opening (v) is shown. In (c), the clitoris is again hidden by labial folds, but the vaginal opening (v) is more obvious. (a-c) are twice their normal size.

FIGURE 3.5: Measurement of the width of the pudendal pad of female marmosets. The adult values are from single measurements of 20 females; for the 53 measurements from young females, 31 were measured once, 11 were measured at two and 11 at three different ages. The curve is fitted by eye.



there was considerable variation, but the adult pudendal pad width of 20.1 ± 0.5mm. was reached by about 425 days of age.

There were no changes evident in the appearance of the mammary glands of female marmosets until after their first pregnancy. In nulliparous females, the small mammaries remained nodular, about 1.0 - 2.5mm. in length and 1.0 - 2.0mm. in basal diameter, identical to the males. However, towards the end of the first pregnancy the mammaries enlarge to a length of 4.0 -7.0mm. and a basal diameter of 2.0 - 3.0mm., similar to multiparous females. Nevertheless, even when enlarged, the mammary glands are normally obscured from view because of their axillary position.

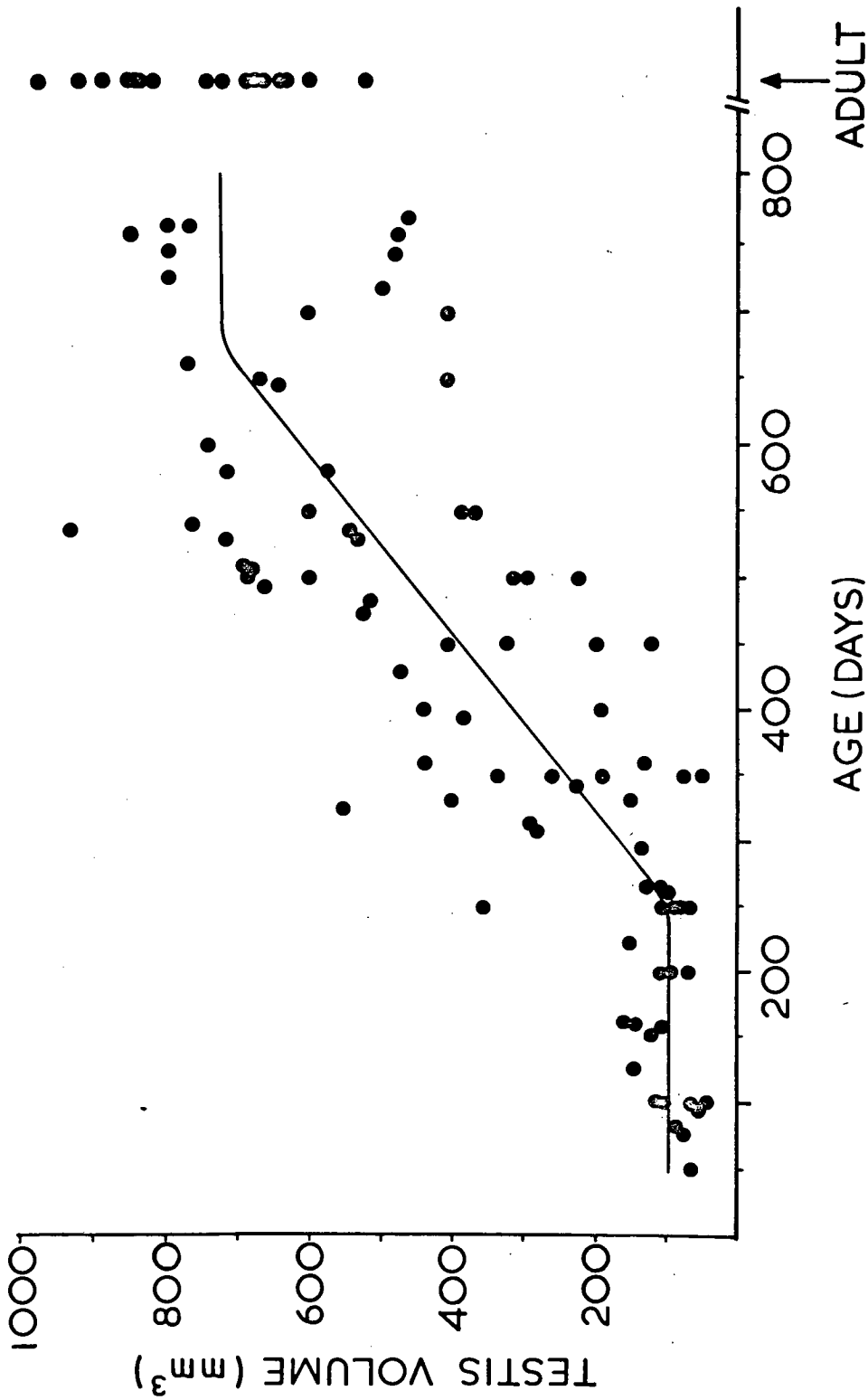
3.3c Testis volume

The volume of the right testis in male marmosets from birth to 800 days old is shown in Figure 3.6. The testes are palpable at birth in the inguinal region and can descend into the scrotum at 50 - 70 days of age. These animals retain the ability to withdraw their testes into the inguinal region throughout life. The testes remain unchanged until 250 days (volume of right testis, 97.6 ± 8.2mm.^{3}; n=18) and then grow rapidly, reaching the adult size of 729.6 ± 25.3mm.^{3} at 650 - 700 days of age. There was considerable variation between animals in the rate of growth, the time at which rapid growth of the testis commenced, and the time at which adult testicular size was achieved.

3.4 Discussion

The marmoset reaches physical maturity relatively rapidly, at an age of 18-24 months, when compared to other primates. This is

FIGURE 3.6: Measurement of the volume of the right testis in male marmosets. The adult values are from single measurements of 20 males; for the 55 measurements from the young males, 27 were measured once, 22 were measured at two and 6 at three different ages. The sigmoid curve is fitted by eye.



a much shorter time than the 5-7 years necessary for the rhesus monkey, Macaca mulatta (van Wagenen and Catchpole, 1956), 4-8 years for the yellow baboon, Papio cynocephalus (Altmann and Altmann, 1970; Lister, 1975), or 12.5-14.5 years for the chimpanzee, Pan troglodytes (Smith, Butler and Pace, 1975), and may be correlated with the marmoset's shorter life-span and smaller body weight (marmoset: 167-360gm., 12 years; rhesus macaque: 4.65-14.5kg., 21.5 years; baboon species: 11-30kg., 28-29 years; chimpanzee: 40.6-48.9kg., 30-38 years; Napier and Napier, 1967).

The identical weight and skeletal development of males and females, and the lack of sexual dimorphism in the adults, is typical of a monogamous species (Short, 1977; Kleiman, 1977; Clutton-Brock, Harvey and Rudder, 1977), and is in agreement with the behavioural evidence for monogamy in the marmoset (Epple, 1967; Rothe, 1975; Stevenson, in press, a). These results are in contrast to those cited by Ralls (1976), who has indicated that marmosets are sexually dimorphic with respect to their ventral sterno-pubic length. However, the sexes only differed by 2% in these latter measurements, and as Hershkovitz (1977) points out, this is probably due to the different shape of the female's pelvis necessary to accommodate the birth of offspring. Hence pelvic measurements may not be indicative of overall size. Hershkovitz (1977) also went on to show that Wettstein's (1963) measurements (cited by Ralls, 1976) were probably taken from immature or underdeveloped adults because of their small skeletal size.

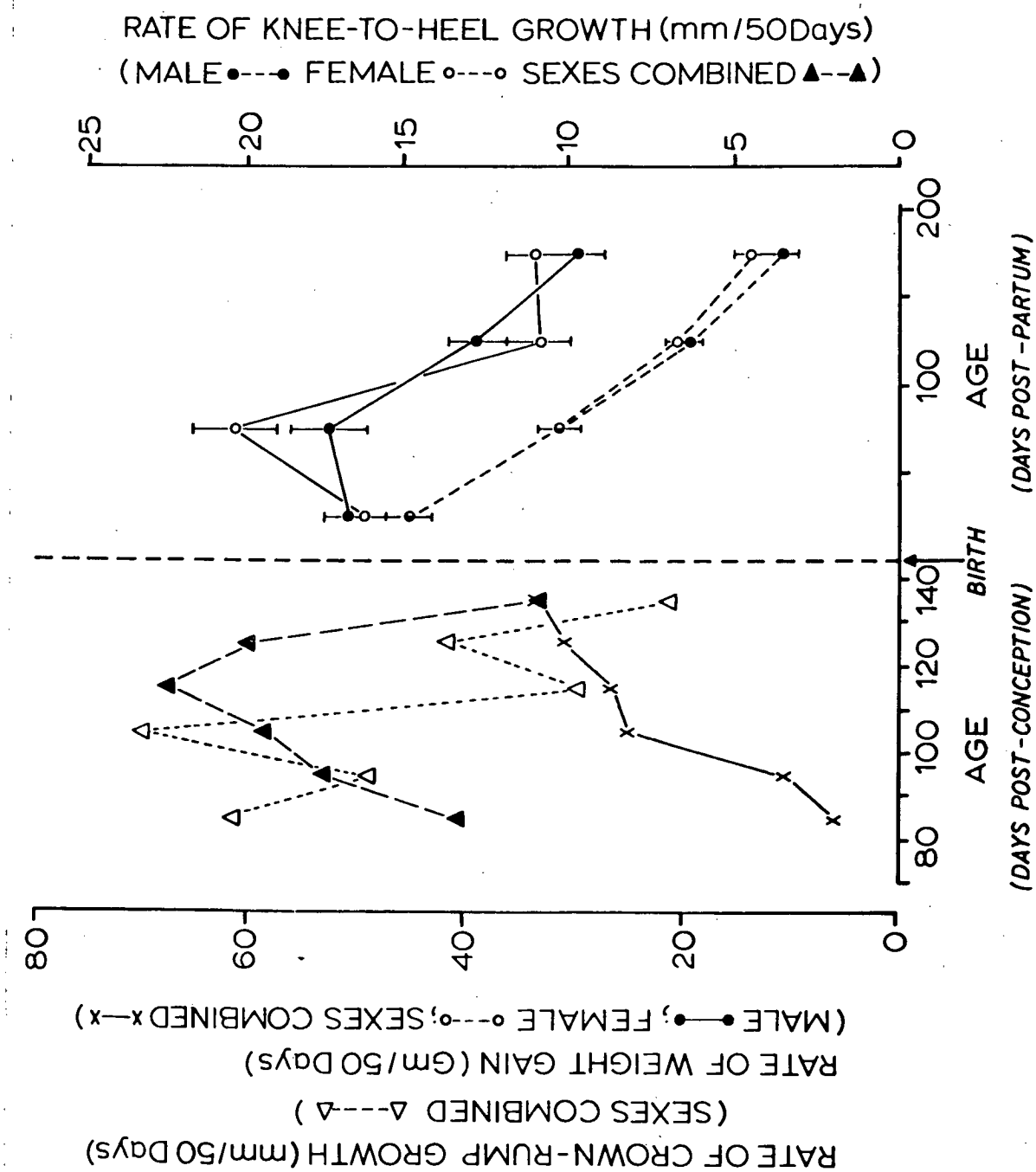
The growth curve of marmoset body weight in our colony (Fig. 3.1) was similar to those reported by Kingston (1975) and Turton et al (1978). The adult body weights are also consistent with those cited by Napier and Napier (1967) and Hershkovitz (1977).

In contrast to the findings of Lucas (1937) and Fess (1975), our colony-bred animals did not grow larger than the captive monkeys originally caught in the wild.

Furthermore, as in the rhesus monkey, chimpanzee and man (Tanner, 1962), birth in the marmoset coincides approximately with the peak rate in growth (Fig. 3.7). However, unlike these other primates, there was no large pubertal growth spurt. It occurs at the same age in both sexes and is only of some significance in the males (Table 3.1). But, as the marmoset is fully grown within 600 days and the rates of growth were calculated every 50 days, the intervals of measurement may have been too large to accurately reflect changes of growth rates during puberty. This may explain the lack of a pronounced pubertal growth spurt and may also be the reason why the small female peak coincides with that of the males and does not precede it, as in other primates (Tanner, 1962). Moreover, in the chimpanzee, the rhesus monkey, and the human, males always have a larger growth spurt at puberty than females and this is attributed to the larger size attained by males after puberty (Tanner, 1962). Perhaps in the marmoset where there is no such sexual dimorphism, the pubertal growth spurt can be expected to be small and equally pronounced in both sexes. There is no pubertal spurt in the rate of knee-to-heel growth because this measurement had previously reached adult proportions.

The external genitalia of young male and female marmosets also showed little difference in appearance (Fig. 3.3 and 3.4), another typically monogamous trait (Short, 1977), and this has led to a 1-2% error in sexing animals at birth. In the development of the mammary glands female marmosets follow a similar pattern to that in other female primates and differ from the human. In primates, the

FIGURE 3.7: Perinatal rates of weight gain (——), knee-to-heel growth (---) and crown-rump growth (····) in male (●) and female (o) marmosets (and both sexes combined). All mean values are converted to rates per 50 days and post-natal values are ± s.e.m. The pre-natal data was calculated from measurements taken by P.L. Chambers (unpublished work) following the hysterotomy of pregnant animals, and triplet sets were omitted from the last point in weight gain because they were significantly smaller than twins at this age. The post-natal data is taken from Figure 3.2.



mammaries develop during the first pregnancy (Milligan, Drife and Short, 1975), whereas in the human the breasts develop at puberty. This suggests that in the marmoset, as in other monkeys and apes, the mammary glands only develop to fulfill their lactational role.

3.5 Chapter summary

(1) The physical development of marmoset monkeys was completed in 18-24 months.

(2) Measurements of body weight and knee-to-heel length were identical for both sexes, typical of growth patterns for a monogamous species.

(3) There was no pronounced pubertal growth spurt.

CHAPTER 4 SEXUAL DEVELOPMENT: ENDOCRINOLOGY, REPRODUCTIVE BEHAVIOUR, FERTILITY AND BREEDING SUCCESS

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4.1 Introduction

Rothe (1975) has described the development of sexual behaviour in young C. j. jacchus. Phillips(1975, 1976a), from pregnancy data in the same sub-species, estimated that females reached puberty by 13-14 months of age or earlier, and Kingston (1975) cited 15 months. From the same type of data, Hampton, Hampton and Levy (1971) reported that female marmoset and tamarin monkeys became pubertal in "less than 2 years" and Wolfe, Deinhardt, Ogden, Adams and Fisher (1975) estimated 20 months. Wolfe et al (1975) noted that the youngest female tamarin to produce young was 17½ months old (Saguinus illigeri). However, the endocrinological and physiological aspects of puberty have not been studied in any marmoset or tamarin monkey.

The hormonal profiles of developing animals are particularly important because full maturation of the hypothalamic-pituitary-gonadal axis is required for successful reproduction (Grumbach, Grave and Mayer, 1974a). With the onset of puberty there is a progressive decrease in sensitivity of the hypothalamic and pituitary negative feedback to gonadal hormones, which results in increased secretion of pituitary gonadotrophins, stimulation of gonadal hormone output and the development of secondary sexual characteristics (Donovan and van derWerff Ten Bosch, 1965; Ramirez and McCann, 1963; Critchlow and Bar-Sela, 1967; Smith and Davidson, 1967, 1968; Davidson, 1969; Ramirez, 1971). In the female primate this is followed, later in puberty, by the maturation of the positive feedback mechanism which controls ovulation. The positive feedback action of oestrogen was not demonstrable in pre-pubertal and pre-menarchial girls (Grumbach, Roth, Kaplan and Kelch, 1974b) and up to the first few months following menarche in female rhesus monkeys (Dierschke,

Karsch, Weick, Weiss, Hotchkiss and Knobil, 1974b). Positive feedback could be demonstrated in these monkeys shortly before ovulatory cycle began, but this was about a year after menarche (Dierschke et al, 1974b).

In this chapter the normal sexual development of the common marmoset monkey will be presented before venturing on to experimental manipulation of development (in Chapter 6). This chapter will therefore collate detailed measurements of plasma hormone levels in intact males and females (as illustrated in Table 4.1), with observations of their reproductive behaviour, fertility and breeding success, in an attempt to gain a comprehensive understanding of their sexual development. These detailed measurements are required because animals of this sub-species exhibit little sexual dimorphism and females neither menstruate nor show any physical signs of oestrus.

4.2 Procedure

4.2a Schedule of collection of plasma samples for radioimmunoassay of hormone concentrations

Oestradiol

Blood samples were collected (as illustrated in Chapter 2, section 2.11) at 15-30 day intervals from females under 200 days of age, and at 2-3 day intervals from females over 200 days, for periods of up to 2 months. Single samples were also taken from females up to 500 days old, and random samples were taken from females over 500 days. Three non-pregnant adult females were sampled at 2-3 day intervals for 1-2 months for comparison (P.L. Chambers, unpublished work). The frequency of adult sampling was sufficient to give adequate coverage of the ovarian cycle lasting 16.4 ± 1.7 days (Hearn and Lunn, 1975).

TABLE 4.1: Plasma hormones measured in developing male and female marmosets

Plasma hormones measured	
Females	Males
Oestradiol	Testosterone
Progesterone	LH
Testosterone	
Luteinising hormone (LH)	

Progesterone

Sampling followed an identical regime to that for oestradiol, except that females over 500 days of age were also bled at 2-3 day intervals for 2-4 months, and seven non-pregnant adult females were used for comparison.

Testosterone

While under 200 days of age, three females were sampled at 15-30 day intervals, and at 30-60 day intervals thereafter. Fifteen other non-pregnant females were sampled at random between birth and 600 days of age.

Males under 200 days old were bled at 15-30 day intervals for 2-4 months and males over this age were bled every 10 days for a similar period. Seven adult males were also sampled at 3-monthly intervals and several consecutive bleeds were taken on each occasion, 2 hours apart.

Luteinising hormone (LH)

Blood samples were collected at about 30-day intervals from males and females under 200 days old and at 30-60 day intervals from animals over 200 days, for a total of 6-12 months. The range of adult values were taken from 6 males and 3 non-pregnant females (J.K. Hodges, personal communication).

4.2b Structure of the different social groups

Young marmosets were housed with their parents until 300-550 days of age, when they were removed to establish breeding groups. Because randomly paired colony-bred male and female marmosets have been slow to breed in other colonies (Kingston, 1975), three options were examined to determine the most efficient system for breeding:

(1) young, unrelated marmosets were placed together to give 4 male-female pairs;

(2) young male or female marmosets were paired with experienced adults of the opposite sex (n=12);

(3) 33 peer groups were established, each comprising 6 unrelated young marmosets (usually 3 male and 3 female). After the initial part of the study, when they were at least 600 days of age, all of the females in social system (1), two in system (2) and three other females were placed with adult, experienced males because they had failed to conceive or raise offspring with their previous partners. Eleven females from social system (3) were also moved because of their failure to breed. However, they will not be included in the following results since they were subordinate females in their peer groups and subjected to reproductive inhibition because of their behavioural status (see Chapter 5).

4.2c Observation schedules of the different social groups

Families

17 family groups were observed for a total of 425 hours in the observation room (as described in Chapter 2).

Pairs

The animals were observed through a one-way mirror in an exercise cage of 1.5 x 1.5 x 1.5m., supplied with branches, ropes etc. Each pair was observed for 2h/week for 10 weeks in the same exercise cage, with five observations at 12:00-14:00h. and five at 14:30-16:30h. The observation times altered each week for every pair. Behavioural recordings were made on an Esterline Angus pen recorder. Vaginal washings were examined daily for the presence of spermatozoa (see

Chapter 2), except during Weeks 3, 4 and 8 of observation. Vaginal washings were also taken after observed copulation.

Observations of the pairs were carried out when the young marmosets were between 400 and 550 days of age, because marmosets are thought to become pubertal at about 450 days of age (Kingston, 1975). Young male-female pairs were established 100 days before observation began. One young female and two young males were each paired with an adult of the opposite sex at the first observation period.

Peer groups

26 of the 33 peer groups were observed for a total of 86 hours, as described in Chapter 2. After every group was set up, vaginal washings from each female were examined daily for at least four weeks or until spermatozoa were identified (whichever was the longer period).

4.2d Reproductive behaviour recorded

All copulations observed in families, pairs and peer groups were recorded, as well as chance observations of any copulations in the home cages. The age at which each colony-bred animal was first observed copulating was also noted. Copulations were identified as "mounts accompanied by pelvic thrusting", as described in Chapter 2.

4.2e Pregnancy detection and spontaneous abortions

Pregnancies were monitored either by radioimmunoassay of plasma progesterone concentrations or by transabdominal uterine palpation. Plasma progesterone concentrations during pregnancy and growth curves for the uteri of marmosets carrying single, twin and

triplet fetuses have been reported previously (Hearn and Lunn, 1975; Hearn et al, 1978). The age of females at the time of their first conception was calculated from the date of birth of the first young in term pregnancies (n=37), assuming a gestation length of 144 ± 2.0 days (Hearn et al, 1978; Chambers and Hearn, in press), or from the uterine diameter when the first pregnancy ended in a spontaneous abortion (n=18). The proportions of spontaneous abortions experienced by colony-bred females during their first to fourth pregnancies were then compared to those experienced by adult wild-caught females during one year (July, 1976 - June, 1977), using the chi-squared test (Siegel, 1956). Data from the 'first' to 'fourth' pregnancies of wild-caught females were not used for comparison with those from colony-bred females because of the dirth of information about their pregnancies before capture, and the emaciated and stressful condition in which the wild-caught animals arrived from South America (Hearn et al, 1975).

4.2f Evaluation of breeding success

In calculating the percentage of offspring weaned and the occurrence of infant deaths, all stillbirths and the number of young in each set in excess of two were excluded because marmosets do not raise more than 2 offspring from each birth without artificial aid (Hearn et al, 1975; Hearn and Burden, in press) Marmosets in our colony are normally weaned by 40 days of age and data from young dying before this age were analysed by using the chi-squared test when comparing different social systems and the Sign test (one-tailed; Siegel, 1956) for the individual pairs or peer groups within each social system. In the latter test, each pair or peer group is given

a score under two different ratings: under rating A (the occurrence of no infant deaths), if no infants have died they score (1), and if any have died they score (0); under rating B (the occurrence of one or more infant deaths), if any infants have died they score (1) and if none have died they score (0). The Sign test is then based on the equation A - B = +1 or -1, for each pair or peer group. If any infants have died, the equation will give them a value of -1, and if none have died, they will be given a value of +1.

4.3 Results

4.3a Changes in plasma hormone levels of young females

There were small fluctuations in plasma oestradiol concentrations following birth (Figure 4.1), but it was not clear whether or not these represented a neonatal surge. After about 200 days of age, levels of plasma oestradiol ranged from undetectable (< 0.2ng/ml) to those equivalent to the pre-ovulatory oestradiol surge of the adult (about 0.8 - 2ng/ml; Hearn et al, 1978).

Fluctuations in plasma progesterone concentrations were relatively small compared to those of oestradiol until females reached 400 days of age and over; values of 20ng/ml or more were then obtained (Figure 4.1b). These high progesterone levels usually follow ovulation in adult females and levels of progesterone in the luteal phase of the adult cycle can vary between 20 and 140 ng/ml for 7-8 days or more (P.L. Chambers and J.P. Hearn, unpublished data). Females also conceived for the first time between 335 and 989 days (Table 4.2), confirming that ovulation could occur from about 400 days onwards. High progesterone concentrations indicative of ovulation could be found in young females remaining with their families (n=5), in pairs (n=7) and in peer groups (n=16).

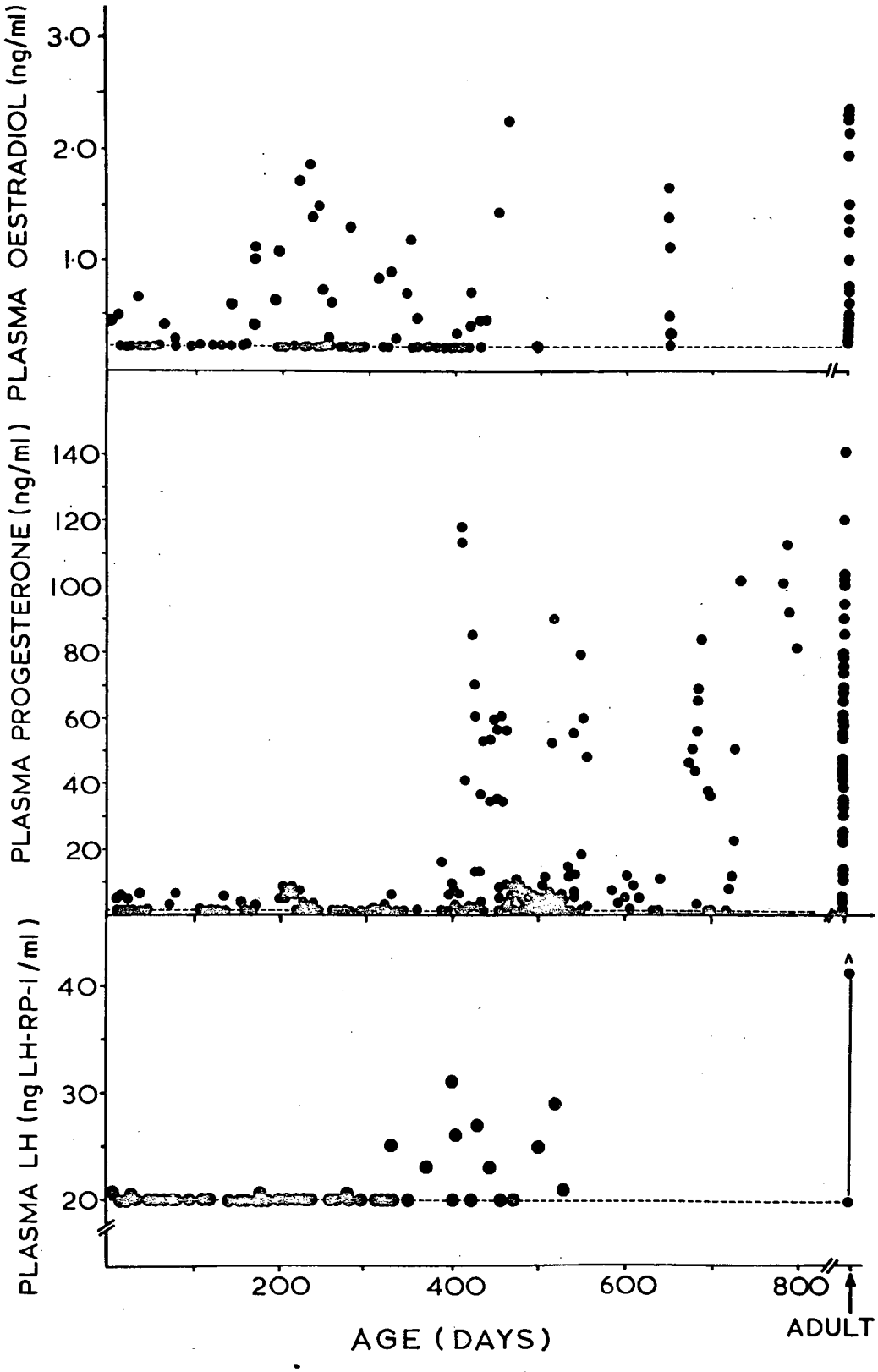


FIGURE 4.1: Plasma oestradiol, progesterone and LH concentrations in female marmosets. Individual sample values are plotted (except for the adult range of LH).

^ = up to 120 ng/ml (mid-cycle peak).

--- = denotes the sensitivity limit of the assays.

Table 4.2: Data pertaining to puberty in marmosets of different social groups

Social Group				Age of males (days)			Age of females (days)			Time from formation of group to conception (days)
Type	Male	Female	No.	At first observed copulation*	When sperm first found in vaginal washings*	When female conceived	At first observed copulation*	When sperm first found in vaginal washings*	When first conceived	
Pair	J	J	1	501	510	-	520	529	-	-
			2	495	-†	680	504	-†	695	377
			3	508	517	-	527	536	-	-
			4	487	536†	567	499	548†	583	266
Pair	J	A	5	499	511	503	(Adult)	(Adult)	(Adult)	28
			6	440	440	470	(Adult)	(Adult)	(Adult)	107
Pairs	A	J	(n=10)	(Adult)	(Adult)	(Adult)	530·0±53·9 TP (335 - 723)	530·2±54·0 TP (335 - 724)	618·2±56·9 Δ (335 - 974)	42.3±12·9 Δ (7 - 125)
Pairs	Ac	J	(n=11)	(Adult)	(Adult)	(Adult)	NR	NR	775·5±47·4 (549 - 989)	35·6±9·9 (2 - 107)
Peer groups			(n=20)	463·2±26·1 ‡ (330 - 742)	476·6±25·1 ‡ (332 - 742)	641·0±31·1 (450-1031)	464·1±19·7 ‡ (340 - 613)	473·9±18·7 ‡ (362 - 621)	568·0±19·7 (382 - 981)	97·4±23·8 (0 - 463)

Values are mean ± S.E.M. (range).
J = young animals; A = adult animals; Ac = adult animals but not the original partner.
NR = data not recorded. TP n = 6. ‡ n = 16. Δ n = 9.

*No data for Pairs 1-4 for 100 days before observation : see text.

†The labia of these females were fused and were artificially opened at 507 and 530 days of age.

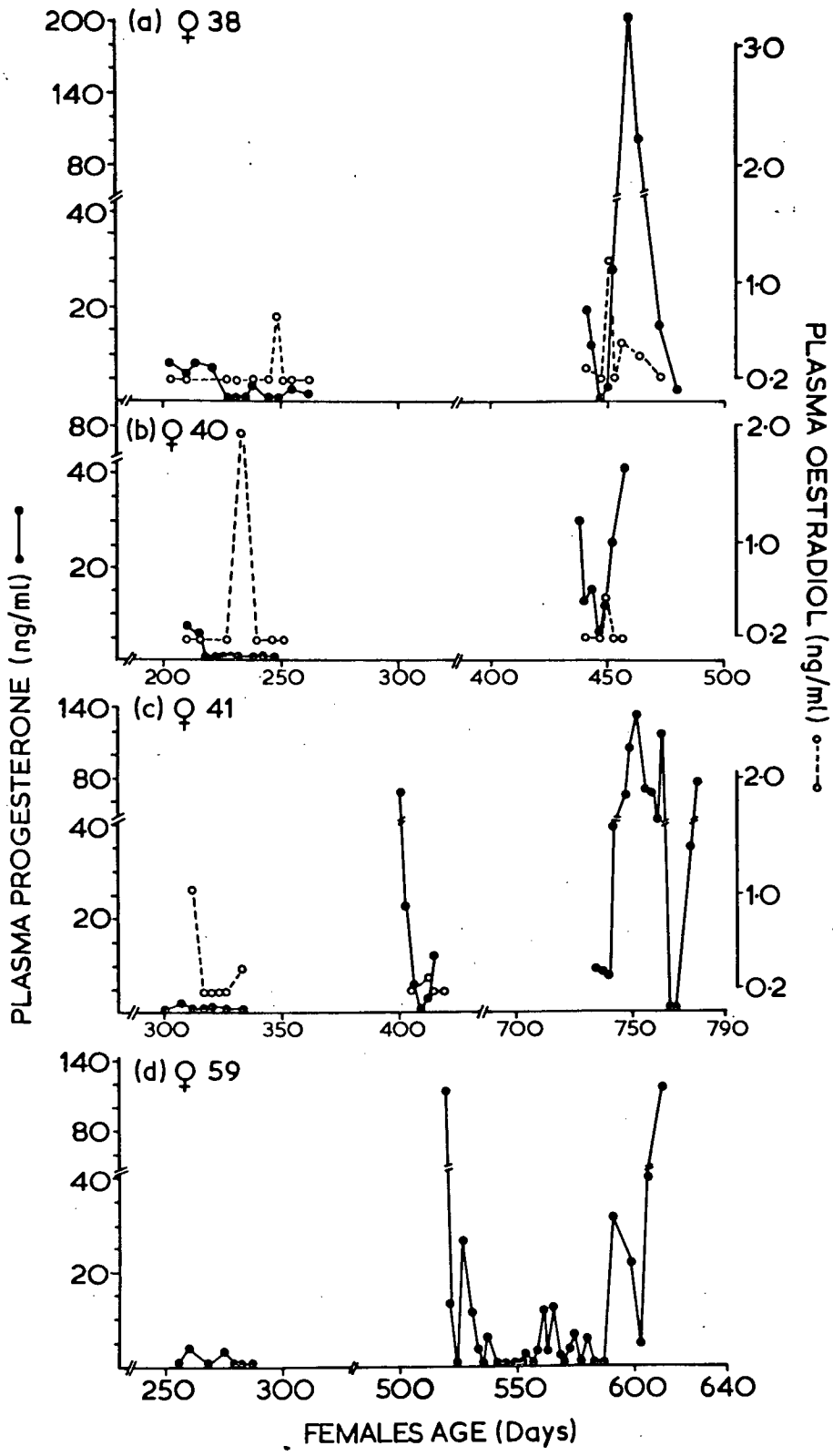


FIGURE 4.2: Plasma oestradiol and progesterone concentrations in four young female marmosets (a) Female 38, (b) Female 40, (c) Female 41 and (d) Female 59.

TABLE 4.3: Duration of ovarian cycles in young female marmosets

Cycle length‡ (days)	Illustration
9,17	♀59, Figure 4.3
10	(c), Figure 5.14 (■ - ■)
29	(a), Figure 5.14
29	(a), Figure 5.15
30	♀38, Figure 4.3
32	♀41, Figure 4.3
34	(c), Figure 5.14 (o - o)

‡ See text.

Now if the relationship between plasma oestradiol and plasma progesterone concentrations is examined in individual animals, no synchrony is achieved between these two hormone levels until females reached 400 days and over (as suggested by their scattergrams Figures 4.1a,b). In three females between 200 and 340 days of age (Figure 4.2a-c), oestradiol levels were found equivalent to those of the pre-ovulatory surge in the adult, but there were no corresponding responses in progesterone levels, indicating that ovulation did not take place. However, by 400-500 days, pre-ovulatory oestradiol peaks could be followed by large increases in progesterone levels, indicative of the adult ovarian cycle (Hearn and Lunn, 1975). Nevertheless, not all females exhibited ovulatory cycles at this age. Providing the females were not pregnant, cyclical variations in plasma progesterone would continue, as illustrated in Figures 4.2(c) and (d) with 500-800 day old animals. Cycle length varied between approximately 9 and 34 days (n=8, Table 4.3) as estimated from the time between either each fall in progesterone to follicular phase levels (below 20 ng/ml), or each rise in progesterone to luteal phase levels (above 20 ng/ml).

Plasma testosterone concentrations were virtually unmeasurable ($\leq$ 2 ng/ml) and never exceeded 3 ng/ml. On the other hand, plasma concentrations of LH were measurable ($>$ 20 ng/ml) from about 350 days onwards (Fig. 4.1c), and were comparable with those observed during the mid-cycle ovulatory peak in adults (J.K. Hodges, unpublished work).

4.3b Changes in plasma hormone levels of young males

Plasma testosterone concentrations were elevated in neonates from days 5 to 100, with peaks of up to 13 ng/ml at about 40 days of age (Figure 4.3). Levels then declined to less than 3 ng/ml and were

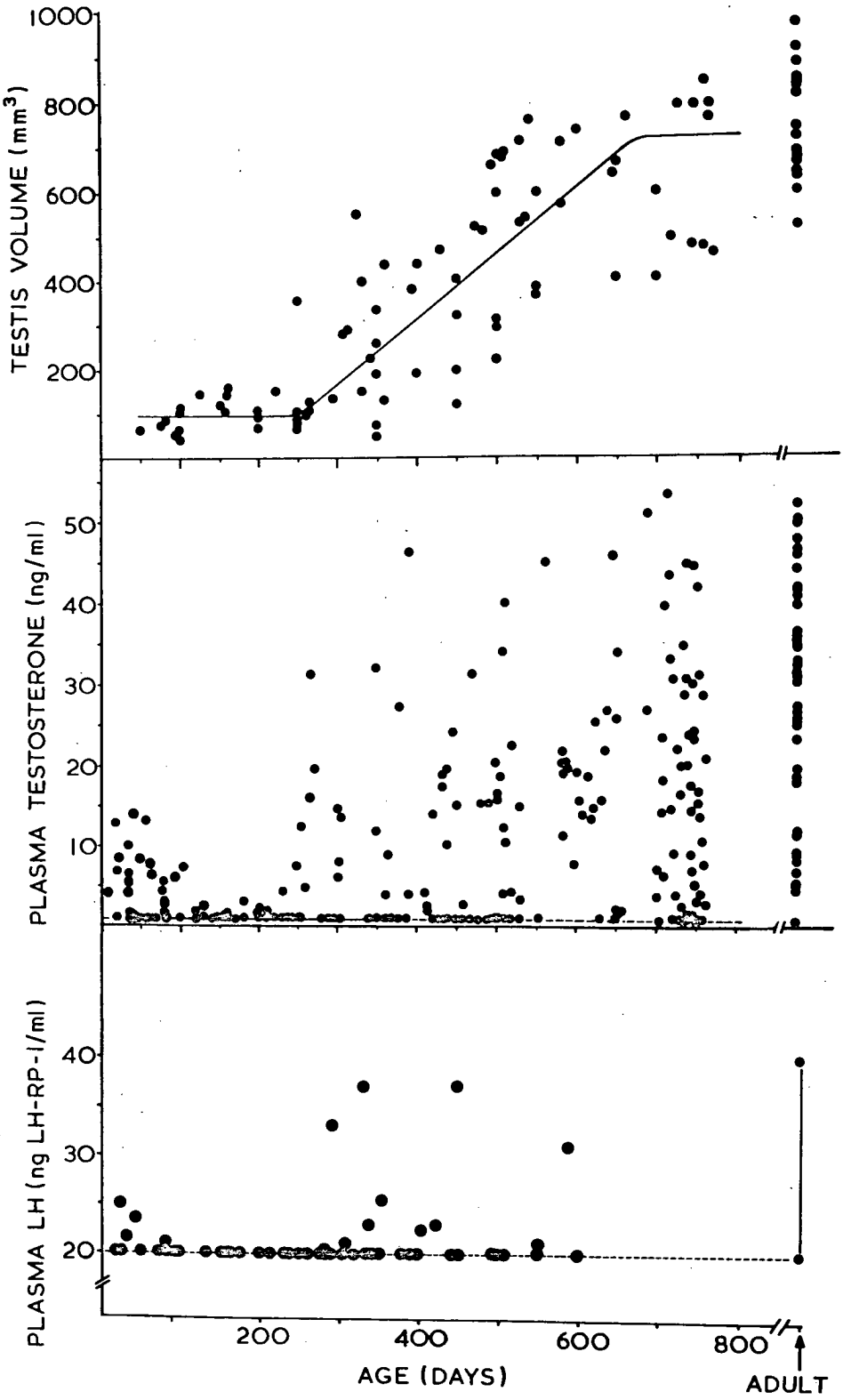


FIGURE 4.3: Measurements of the volume of the right testis, plasma testosterone and LH concentrations in male marmosets. Individual sample values are plotted (except for the adult range of LH). The sigmoid curve is fitted by eye.

--- denotes the sensitivity limit of the assays.

unmeasurable while the volume of the right testis remained below 174.2 mm^3. At about 250 days, testosterone levels rose sharply, coincident with the increase in testicular volume, to attain levels of 30-50 ng/ml, which were in the upper part of the range for adult males.

As illustrated in Figure 4.4, testosterone is secreted from the testes in a pulsatile fashion. Such a pattern is not obvious in the neonatal period because of the large sampling intervals employed with these small animals, but after the quiescent juvenile period, pulsatile fluctuations in plasma testosterone concentrations become obvious. Not all males exhibit large changes in their testosterone levels between 250 and 350 days of age, but by 500 days all exhibit the typical adult pattern (as illustrated by two examples in Figure 4.4). The pulses in testosterone concentration appear even more dramatically if the sampling interval is reduced to 2-3 days, as shown by two males at 750 days of age.

Plasma LH concentrations followed a similar pattern to those of testosterone (Figure 4.3). Elevated neonatal levels could be found up to 100 days of age and then levels became undetectable until about 300 days when concentrations similar to those in intact adults appeared (Hodges, 1978).

4.3c Reproductive behaviour and fertility

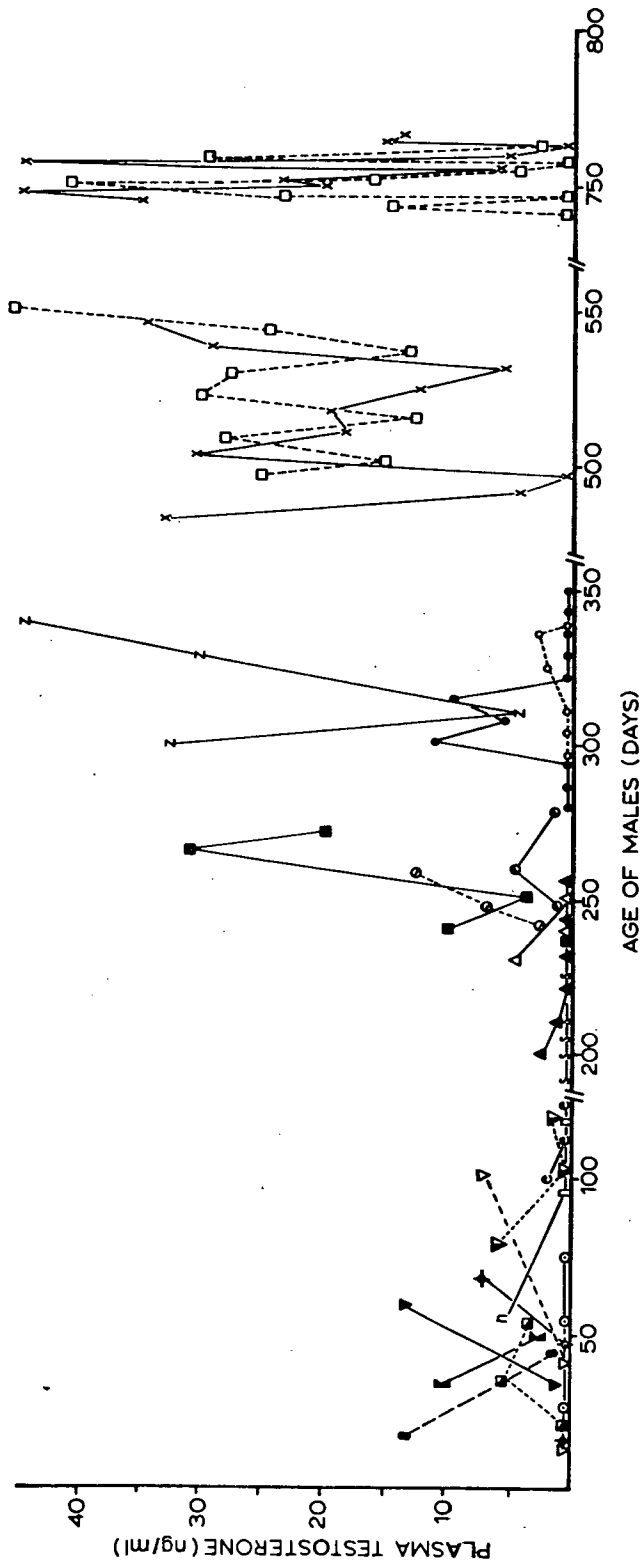
Families

Sexual behaviour was rarely displayed by offspring remaining within their family groups. Juvenile males in only 4 of the 17 families occasionally mounted their mothers and exhibited pelvic thrusting from 250 days of age. This is the time when plasma testosterone

FIGURE 4.4: Plasma testosterone concentrations in 20 young male marmosets.

Male no.	Symbol	Male no.	Symbol
2	▼	39	o
3	□	43	+
5	X	47	Δ
7	●	48	▲
12	0	49	S
13	◪	61	e
16	◧	62	▼
17	⧓	67	n
29	Z	68	■
35	⊙	70	⊘

FIGURE 4.4


PLASMA TESTOSTERONE (ng/ml)
10
20
30
40
AGE OF MALES (DAYS)
50
100
200
250
300
350
500
550
750
800

levels and testis volume begin to increase (Figure 4.3). There was no evidence that these younger males ejaculated. However, two of them exhibited penile erection, and five other males, aged 300-510 days old, ejaculated normal, motile spermatozoa when tested with unfamiliar females outwith the family (see Chapter 5).

In two other families, the adult males were observed mounting their daughters (350-400 days of age). The mounts were terminated violently by the daughters within 2-3 sec. and neither daughter was found to have ovulated, as determined by their low plasma progesterone concentrations (< 10 ng/ml) during the subsequent 10 days.

Pairs and peer groups

Details of the reproductive activity and fertility of the marmosets in the three types of social group are summarised in Table 4.2. Young males were first observed copulating from 330-742 days of age with females from 335-613 days of age, and adult females. The earliest conception of a female housed with a juvenile male occurred when he was 450 days of age, and the youngest female to conceive was 335 days old.

When this data relating to puberty was plotted graphically (males: Figure 4.5; females: Figure 4.6), the median age of 400-500 days in both sexes for the first observed copulation and the first detection of sperm in vaginal washings was different to that of 500-600 days for achieving conception. Moreover, young males were observed copulating in their pairs or peer groups 0-49 days before spermatozoa were present in vaginal washings, a significantly longer gap than that of 0-2 days observed with adult males ($p = 0.0043$; Mann-Whitney U test, one-tailed; Siegel, 1956). Adult males with

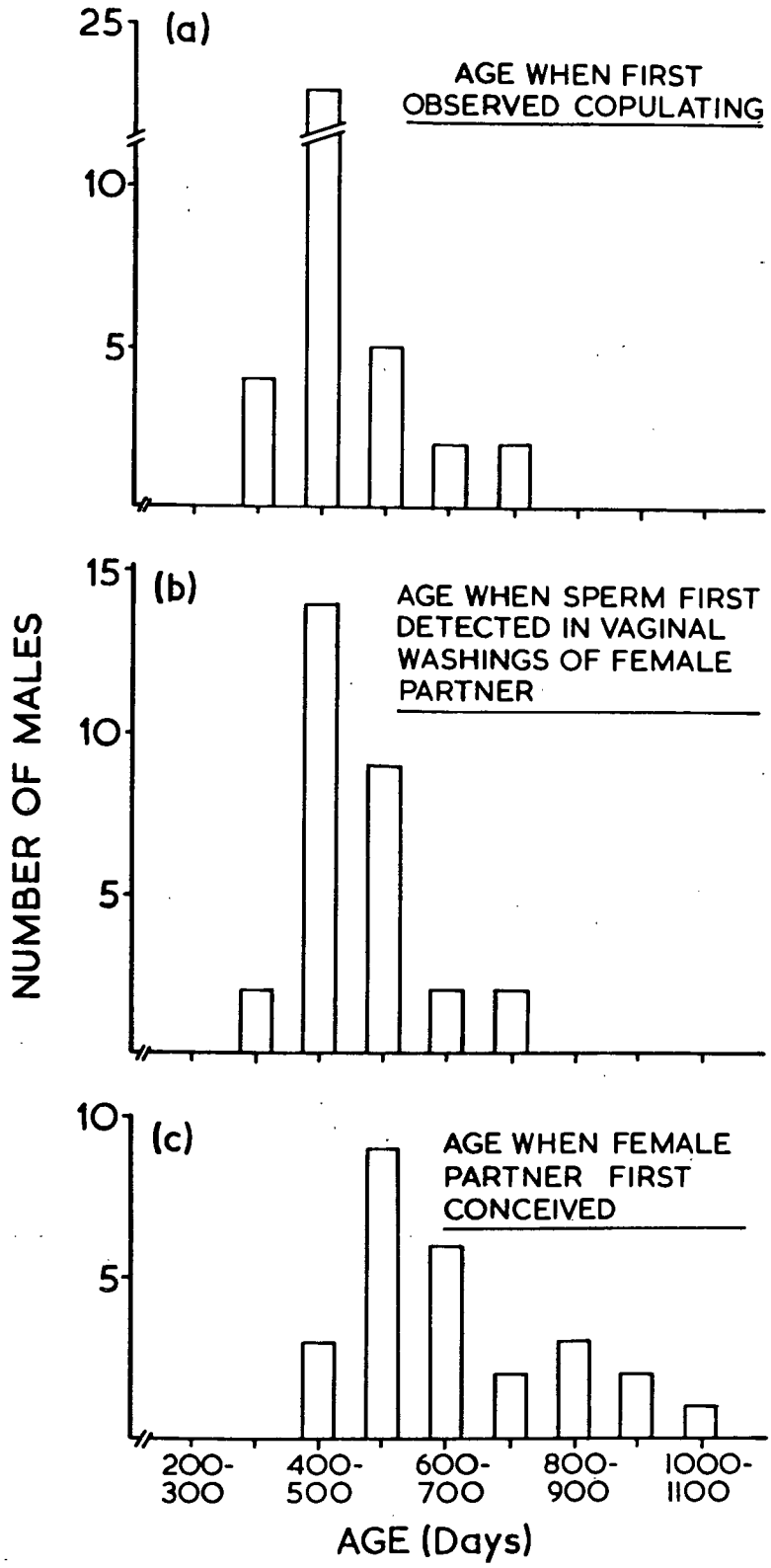


FIGURE 4.5: Data pertaining to puberty in young male marmosets:

(a) the age when they were first observed copulating,

(b) the age when their spermatozoa were first detected in the vaginal washings from their female partner,

(c) the age when their female partner first conceived.

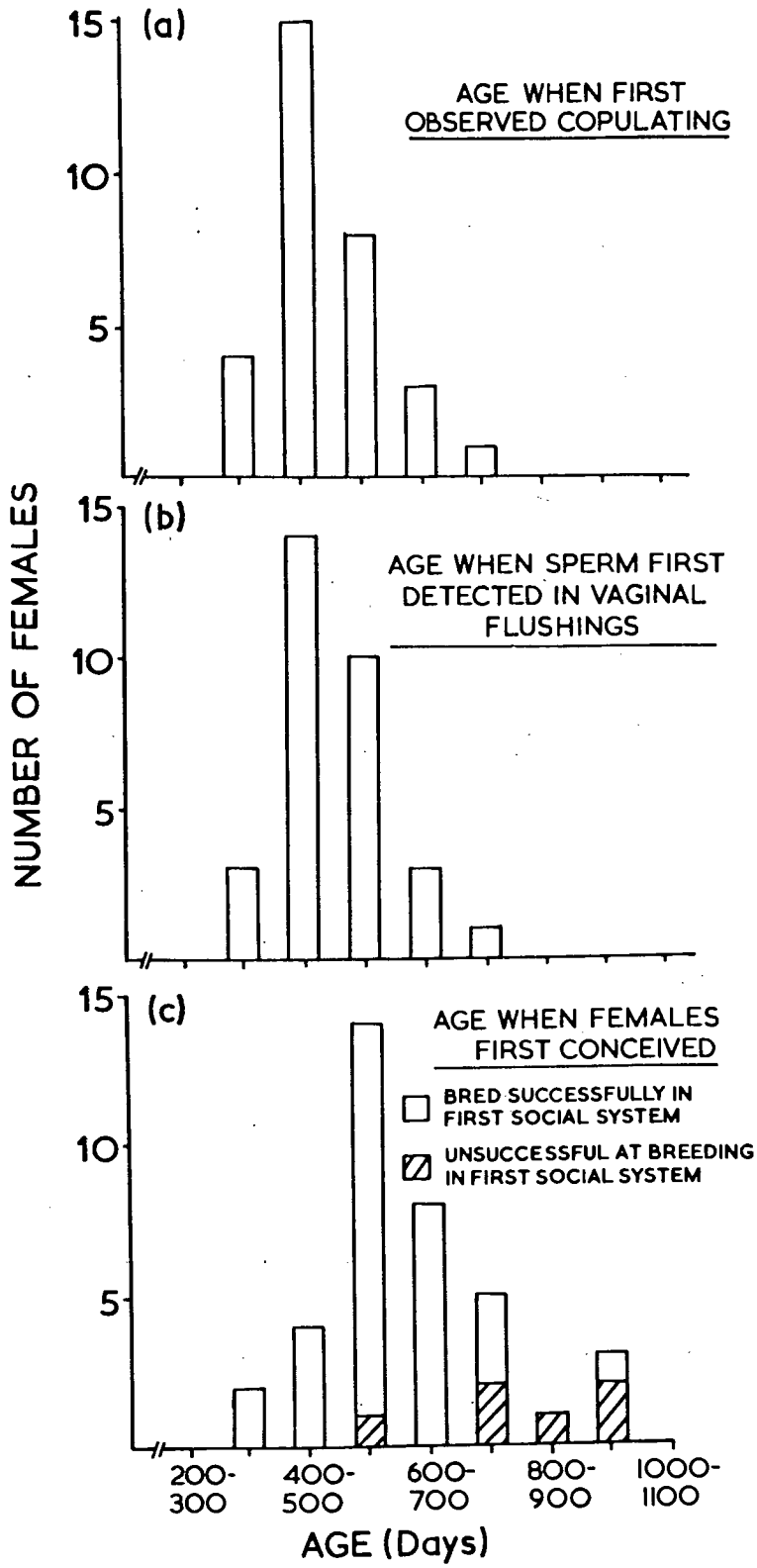


FIGURE 4.6: Data pertaining to puberty in young female marmosets:

(a) the age when they were first observed copulating,

(b) the age when spermatozoa were first detected in their vaginal washings,

(c) the age when they first conceived.

re-partnered females were not considered. Young males were also observed copulating 0-461 days before the first conception was noted (Table 4.2). However, young and adult males did not differ in the time taken between the first finding of spermatozoa in their females' vaginal washings and the first pregnancy. There was no difference (in qualitative terms) between the morphology, number or motility of spermatozoa from young or adult males. Notably, in 43-50% of young animals paired together, young males paired with adult females and peer groups, females took over 100 days to conceive for the first time after the pairs or groups were set up. However, 80-90% of young females paired initially with adult males or removed from their original partners and paired with experienced adult males conceived within 70 days.

4.3d Breeding success of the different social groups and the different rates of spontaneous abortion

In the pairs, all adult females and 11 out of the 14 young females became pregnant, but only the experienced adult females reared almost all of their young (Table 4.4). In pairs with young males, one of the young females spontaneously aborted and the other abandoned her young within 2 days of birth. Plasma progesterone concentrations remained low in one of the remaining two young females (< 10 ng/ml), indicating that she did not ovulate. In pairs with adult males, 8 out of 10 young females managed to raise offspring, one spontaneously aborted and the other failed to ovulate. Young females, aged about 600 days and over, successfully raised their second and subsequent sets of offspring if they were removed from their original partners and paired with experienced adult males (Tables 4.2 and 4.4). Pregnancies in the peer groups were similarly successful (Table 4.4).

TABLE 4.4: Data pertaining to pregnancy and survival of offspring from marmosets in different social groups.

Type	Male	Female	No. of groups	No. of pregnant females	No. of pregnancies	No of. spontaneous abortions	No. of young born	Corrected no. of young born	Young weaned (%)	p_1 ++	p_2 ++
Pair	J	J	4	2	2	1	2	2	0 (0)	-	-
Pair	J	A	2	2	6	0	17	12	12 (100)	-	NS
Pair	A	J	10	9	15	6	18	17	10 (59)	< 0.05	< 0.001
Pair	Ac	J	11	11	34	19	37	25	14 (56)	< 0.01	< 0.001
Family pair	A	A	21	21	NA	NA	227	182	159 (87)	NS	-
Peer group			20	20	55	22	72	60	47 (78)	NS	NS

J = young animal; A = adult animal; Ac = adult animal but not the original partner.
NA = data equivalent to that for the other social groups not available.
Excluding all stillbirths and young in excess of two in each set; see text.

++ Probability values from the chi-squared test (df = 1), Siegel (1956).
p_1 = Comparison with the proportion of offspring weaned by adult females paired with young males.
p_2 = Comparison with the proportion of offspring weaned by adult females paired with adult males.

NS = Insignificant probability values from the chi-squared test ($p > 0.05$).

Of the breeding systems attempted here, only young animals randomly paired together were totally unsuccessful in rearing any offspring, partly due to failure to conceive (Table 4.2 and 4.4). The most successful combination was an adult female paired with a young male, raising a similar proportion of offspring to the adults in family pairs (Table 4.4). Nevertheless, while young females with peer groups, young females with adult males and young females removed from their partners and paired with experienced adult males did not wean all their offspring, only the young females in pairs raised a significantly smaller proportion than the adult females (Table 4.4). However, there was only a significant occurrence of infant deaths among the latter type of pairing (Table 4.5).

There was a much higher number of spontaneous abortions (48) in the 1-6 pregnancies of the 34 young females than with the two experienced adults partnered by young males (0 in 6 pregnancies, Table 4.3), or the other 21 adults monitored over one year (9 in 39 pregnancies, Table 4.6). Of the first pregnancies of young females, only 52% survived to term (Table 4.6), and only 58% of the offspring were weaned (Table 4.7). The results from these first pregnancies were significantly worse than those from adult females paired with adult males. However, in their second and subsequent pregnancies, their proportion of offspring weaned approached that of the adults (Table 4.7), but without any improvement in the rate of spontaneous abortions (Table 4.6).

4.4 Discussion

4.4a Puberty

In marmoset monkeys, the endocrinological and behavioural aspects of sexual development are completed within 18-20 months,

TABLE 4.5: The occurrence of infant deaths among different social groups of marmosets

Type	Male[+]	Female[+]	No. of groups	Rating of infant mortality before weaning (40 days)[++]	
				No deaths	One or more deaths
Pair	J	J	2	0	1
Pair	J	A	2	2	0
Pair	A	J	10	3	4
Pair	Ac	J	11	1	7*
Family pair	A	A	21	12	9
Peer group			20	8	8

[+] Nomenclature given in Table 4.4.

[++] Explanation given in text; see Procedure section.

* p = 0·035 (Sign Test).

TABLE 4.6: A comparison of the proportion of offspring weaned by wild-caught adult females with that weaned by young colony-bred females in their first four pregnancies.

Female	Pregnancy No.	No. of females	No. of young born	Corrected no. of young born ‡‡	Young weaned (%)
Wild-caught	All	21	227	182	159 (87)
Colony-bred	1st	20	48	38	22 (58)***
Colony-bred	2nd	16	37	31	23 (74)
Colony-bred	3rd	8	15	12	9 (75)
Colony-bred	4th	6	13	12	9 (75)

‡‡ Excluding all stillbirths and young in excess of two in each set; see text.

*** $p < 0.001$ ($\chi^2 = 18.72$; 1d.f) when compared with wild-caught females.

TABLE 4.7: A comparison of the annual spontaneous abortion rate of wild-caught females with that of young colony-bred females during their first four pregnancies.

Female	Pregnancy no.	No. of females	No. of spontaneous abortions (%)
Wild-caught	1976-1977‡‡	21	9 (23)
Colony-bred	1st	40	19 (48)**
	2nd	26	10 (38)
	3rd	16	8 (50)*
	4th	11	5 (45)

‡‡ All pregnancies in non-experimental wild-caught females between July 1976 and the end of June 1977 (data equivalent to that for colony-bred females not available).

* $p < 0.05$, ** $p < 0.02$; (χ^2 test; 1d.f.) when colony-bred females were compared with the wild-caught.

a similar time to the 18-24 months taken to reach physical maturity (Chapter 3). Whereas these monkeys develop more rapidly than say a more conventional laboratory primate such as the rhesus monkey, M. mulatta (5-7 years; van Wagenen and Catchpole, 1956), they still retain the typical primate characteristic of a delayed puberty when compared to other mammals of a similar size, such as the rat, which reaches puberty within 2-3 months of age (Tanner, 1962).

Puberty in the marmoset is best described as a period rather than a point in time. In the male it encompasses the increase in plasma testosterone levels and testicular size, and the development of copulatory behaviour which accompany the appearance of spermatozoa, i.e. between 250 and 400-500 days of age. In the female it covers the increase in plasma oestradiol and progesterone levels which accompany the appearance of ovulatory cycles, and the development of copulatory behaviour, i.e. between 200 and 400-500 days. There was therefore no major difference between males and females in the age at which they reached puberty. No clear relationship between body weight and pubertal age was achieved in female marmosets, in contrast to that found in rhesus monkeys (Wilen, Goy, Resko and Naftolin, 1977) and women (Frisch and Revelle, 1970), because females did not exhibit menarche and could be reproductively inhibited by dominant females (see Chapter 5).

4.4b Endocrinological and behavioural aspects of female marmoset sexual development

Indicative of some ovarian activity occurring at least 200 days before the first ovulation took place, plasma oestradiol levels in young females reached pre-ovulatory peak values of the adult

females without any corresponding responses in plasma progesterone or LH (Figures 4.1 and 4.2). In this respect, the female marmoset may demonstrate similar evidence of phases of follicular development and oestrogen secretion in the ovary as are observed before menarche in the female chimpanzee (Tutin, 1976; Short, 1976b) and the human female (Adamopoulos, Loraine, Lunn, Mackay and Forfar, 1974; Faiman and Winter, 1974; Faiman, Winter and Reyes, 1976; Brown, Harrison and Smith, in press). On the other hand, as female marmosets do not exhibit menarche, such fluctuations in their oestradiol levels may also correspond to the anovulatory cycles observed following menarche in female talapoin and patas monkeys (Miopethecus talapoin and Erythrocebus patas; Rowell, 1977), mangabeys and ceropithecus monkeys (Gautier-Hion and Gautier, 1976), rhesus monkeys (Corner, 1923; Dierschke, Weiss and Knobil, 1974a), chimpanzees (McGinnis, 1973; Tutin, 1976; Short, 1976b) and young girls (Doring, 1969; Adamopoulos et al, 1974; Faiman and Winter, 1974; Faiman, Winter and Reyes, 1976; Brown, Harrison and Smith, in press). Whatever the explanation, maturing female marmosets display ovarian activity long before they become fertile, typical of many Old World monkeys, apes and man.

When ovulatory cycles did occur (as estimated from elevated progesterone levels; section 4.3a) their length was very variable between animals (from 9 to 34 days), and was unlike the estimate of 16.4 ± 1.7 days for the marmoset cycle (Hearn and Lunn, 1975). This is not surprising as the cycle length in young adolescent girls is known to be irregular (Adamopoulos et al, 1974). The short cycles of 9 and 10 days may have been curtailed as they occurred at the time of peer group formation. Cycles have been shortened by fighting in

female Gelada baboons, Theropithecus gelada (Dunbar and Dunbar, 1977). The long cycles (29-34 days) might depict pregnancies that spontaneously terminated before they were detected by uterine palpation (e.g. the results from the SHPT pregnancy test kit; Lunn, Hobson and Hearn, in press), suggesting that the age when females first conceived may be an overestimate. Some of the long cycles might also be prolonged by repeated blood sampling (e.g. Hearn and Lunn, 1975).

4.4c Endocrinological and behavioural aspects of male marmoset sexual development

Developing male marmosets exhibit elevated neonatal levels of plasma testosterone, as do male pig-tailed and rhesus macaques (M. nemestrina and M. mulatta; Robinson and Bridson, 1978) and newborn human males (Forest, Cathiard and Bertrand, 1973 a,b). In male marmosets, this is mirrored by elevated neonatal levels of LH (Figures 4.2 and 4.3). Newborn boys also exhibit significantly higher levels of LH than girls (Faiman, Reyes and Winter, 1974; Forest et al, 1974a). The function of this neonatal hormonal surge in males is still not fully understood, but is thought to be partly involved in the maturation of hypothalamic-pituitary-gonadal axis, which controls the whole pattern of hormonal secretion and reproductive function (Forest, Cathiard, Bourgeois and Genoud, 1974 a; Faiman, Reyes and Winter, 1974; Forest, 1975). The second rise in testosterone levels at about 250 days of age is much steeper, and follows a similar pattern to that seen in the chimpanzee (Martin, Swenson and Collins, 1977) and in the human (Faiman and Winter, 1974) at the onset of puberty. All male marmoset sexual behaviour occurs after this later rise in their testosterone levels.

The rising testosterone levels in adolescent male marmosets show none of the seasonal fluctuations of the adolescent rhesus monkey (Rose, Bernstein, Gordon and Lindsley, 1978) and more closely resemble the steadily progressive rise found in the developing human (e.g. Faiman and Winter, 1974). Adult male marmosets also show a lack of seasonal variation in testosterone levels (S.F. Lunn, unpublished data) unlike the mature rhesus male (Gordon, Rose and Bernstein, 1976; Michael and Bonsall, 1977).

4.4d The contrasting development of sexual behaviour between monogamous and polygamous monkeys

Unlike the developing young of Old World monkeys and apes, young male and female marmosets display hardly any sexual behaviour in their natal family groups. Similar observations of common marmosets were also made by Rothe (1975) and by Epple (1975) on the closely related monogamous tamarin monkeys. Sexual behaviour only becomes more frequent if either young animals are removed from their families and set up in pairs or peer groups with unrelated animals, or if one of the parents dies (Rothe, in press). This, of course, is in contrast to the precocious sexual behaviour displayed by infant and juvenile rhesus monkeys (Goy, Wolf and Eisele, 1977) and chimpanzees (C.E.G. Tutin and W. McGrew, personal communication). However, young captive rhesus monkeys show increased sexual behaviour when transferred into peer groups (Goy, 1978). Perhaps this behavioural difference reflects the restriction of most of the reproductive behaviour amongst monogamous monkeys to the breeding pair, in contrast to the promiscuous behaviour observed with the polygamous macaques and chimpanzees. Observations of groups of unrelated marmosets

reinforce this suggestion to some extent, as the breeding pair copulate most frequently and attempt to restrict copulations by other group members (see Chapter 5; Epple, 1975; Rothe, 1975). Nevertheless, the breeding pairs of such groups do not copulate as exclusively as parents in family groups.

4.4e The relationship between puberty, fertility and sexual maturity in marmoset monkeys

If sexual behaviour and fertility in young marmosets is examined further, a distinction appears between the age at puberty (400-500 days) when most animals are mating (75% of males; 61% of females), males can ejaculate spermatozoa and females can ovulate, and the age of sexual maturity (500-600 days) when conceptions more frequently occur (Figures 4.5 and 4.6; Table 4.2). Also young females experienced more spontaneous abortions than adult females and raised fewer of their offspring from the first pregnancy (Tables 4.6 and 4.7). Asdell (1946) made a similar distinction between puberty and sexual maturity for rodents, sheep, pigs and other primates. There are anecdotal reports of marmosets conceiving as early as 300 days of age, but these pregnancies usually ended in abortions or the young were not reared successfully. The annual rate of spontaneous abortions with experienced breeding females (23%), was similar to that of 21% for another C. j. jacchus colony (Hampton, Hampton and Levy, 1971). However, both these may be underestimates because at least 50% of human pregnancies are now thought to spontaneously abort (Roberts and Lowe, 1975; Cutright, 1975; Tanner, 1978).

There are several possible explanations for this gap in age between attaining puberty (the capacity to reproduce) and attaining

sexual maturity (actually reproducing). Firstly, young males may not achieve intromission or ejaculation when mounting, especially since they took a significantly longer time to inseminate their female partners than adult males. Secondly, even if young males copulate successfully, they may not copulate with sufficient frequency around the times of ovulation for conceptions to occur. However, sperm maturation is not indicated as a contributory factor because there was no difference between young and adult males in the time taken for their females to conceive following insemination. Young females may also contribute to the delay of the initial conception, since they may ovulate infrequently, or at irregular intervals. Ovulatory cycles are known to be irregular in young adolescent girls (Adamopoulos et al, 1974; Brown et al, in press).

4.4f The effects of reproductive experience and different social groups on fertility and breeding success of marmoset monkeys

Higher percentages of offspring were successfully raised by adult females paired with either young or adult males than by any other group combination (Table 4.3), suggesting that for rearing offspring, maternal age and experience might be more important than paternal. Confirming this suggestion both combinations of young females paired or re-paired with adult males weaned a much smaller proportion of their offspring than adult females (Table 4.4). Young females also raised fewer of their offspring from their first pregnancies, but not from the second or subsequent pregnancies (Table 4.6). Epple (1978) and Ingram (in press) have also found maternal experience important in rearing young marmosets and Drickamer (1974) has found a similar result with rhesus monkeys. The importance of

maternal experience was again suggested by the significantly higher numbers of infant deaths among pairs of experienced adult males with re-partnered young females (Table 4.5). Perhaps young females that have difficulty in conceiving or raising young with their first partner will always be poor mothers.

Young animals were more efficient at breeding and rearing offspring in peer groups than in pairs and this may stem from three causes. Firstly, the peer groups introduce an element of mate selection, with competition between unrelated animals (see Chapter 5). Secondly, as the young animals are separated from their families during puberty, the peer groups may provide an adequate substitute for further socio-sexual development lacking in the pairs. Rhesus monkeys can be raised from infancy in peer groups with little apparent social deficiency (Hinde, 1974). Finally, the peer groups provide more animals to share in carrying and caring for the infants. However, the behaviour of common marmosets in the wild has only recently been studied (Stevenson, in press,a) and no information is yet available about how new breeding groups are formed naturally. Nevertheless, the movement of juveniles between social groups may prove to be an equivalent socializing process to that used in our laboratory. Recent studies on the closely related tamarin monkey, Saguinus oedipus, show that there may be considerable interchange of older juveniles between social groups, although the adults remain in the same groups (Dawson, 1976, 1978; Neyman, 1978).

4.5 Chapter summary

(1). Endocrinological and behavioural maturity were reached within 18-20 months of age in male and female marmosets.

(2). Because animals of this sub-species exhibit little sexual dimorphism and females neither menstruate nor show any obvious physical signs of oestrus, detailed measurements of plasma sex hormone levels and reproductive behaviour were required to act as criteria of development.

(3). Females ovulated from about 400 days of age, but showed ovarian activity (i.e. measurable oestradiol 17-β in plasma) up to 200 days previously.

(4). Males experienced elevated neonatal levels of plasma testosterone and LH from 5-100 days, and their testosterone levels rose again at 250 days of age, climbing sharply into the adult range.

(5). The majority of males and females were copulating by 400-500 days of age.

(6). Young male and female marmosets displayed little sexual behaviour in their family groups, unlike the precocious copulatory behaviour seen in macaques and chimpanzees.

(7). In both sexes the age of puberty (400-500 days) and that of sexual maturity (500-600 days) can be separated.

(8). Young females experienced more spontaneous abortions and raised fewer of their offspring from their first pregnancies than adult females.

(9). Maternal age and experience was more important than paternal for the successful rearing of offspring.

(10). Young marmosets in peer groups were more efficient at breeding than in randomly-selected pairs. Hence peer group formation has become part of the routine management to maintain our self-sustaining colony.

CHAPTER 5. DOMINANT-SUBORDINATE RELATIONSHIPS AND THE REPRODUCTIVE SUCCESS OF YOUNG MARMOSET MONKEYS

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5.1 Introduction

5.1a Dominant-subordinate relationships and reproductive success in primate societies

Emulating precedents set in the 1930's and Schjelderup-Ebbe's (1935) conception of a 'peck-order', many studies of monkey social behaviour have defined status in terms of dominant-subordinate relationships. Animals whose behaviour was not restricted by others were called dominant; those whose behaviour was restricted and who showed submission, subordinate (Zuckermann, 1932; Maslow, 1936; Carpenter, 1942; Chance, 1956; Bernstein, 1970; Clark and Dillon, 1973; Hausfater, 1975; Deag, 1977). In many studies, pairs of monkeys (otherwise housed singly) were tested in all possible combinations. Such animals and those kept in groups typically displayed a hierarchical pattern of dominant-subordinate relationships. In field studies, mostly of ground-living monkeys, similar principles were applied and dominance was considered a fundamental feature of group organisation, determining many aspects of behaviour (Zuckermann, 1932; Carpenter, 1942). The relationship between dominance and reproductive success has since been of considerable theoretical interest, with an extensive literature reporting its existence among males in many species (see review by Wilson, 1975).

However, in many laboratory studies, describing the hierarchy became the primary objective, while the evolutionary implications of the behaviour were mostly ignored. It was therefore not surprising that the social dominance theory as applied to monkeys came under attack (Gartlan, 1964, 1968; Rowell, 1966, 1974; Bernstein, 1970). There had been no agreement on the definition of dominance and the behaviour required to measure it (Gartlan, 1964). Poor correlations

sometimes appeared between rank and the distribution or frequency of other behaviour conventionally associated with dominance, such as access to food and water, priority of access to receptive females, grooming and mounting (Rowell, 1966; Bernstein, 1970; Eaton, 1974; Richards, 1974; Syme, 1974). These poor correlations brought into question the predictive value of rank from one social situation to another and the relationship between rank and reproductive success. In addition, careful observations revealed that the subordinate and not the dominant played the key role in determining the dominant-subordinate relationship (Rowell, 1966; Chance, 1967), in contrast to earlier impressions. Finally, hierarchies were always found in captive groups of primates, whereas in wild arboreal primates (Rowell, 1971; Jolly, 1972; Poirier, 1974) and some wild olive baboons, Papio anubis (Rowell, 1967), agonistic behaviour (e.g. threat, attack, avoidance, submission) was infrequent and hierarchies were not obvious. This suggested to Rowell (1967) and Gartlan (1968) that hierarchical formation was related to 'stress' in captivity, and they were supported by the many field studies in which wild primates with hierarchies also showed exaggerated agonistic behaviour because of artificial feeding.

Nevertheless, this challenge to the concept of hierarchies was countered. Firstly, definite hierarchies based on clearly agonistic behaviour have been found in wild vervet monkeys, Ceropithecus aethiops (Struhsaker, 1967), yellow baboons, Papio cynocephalus (Hausfater, 1975) and Barbary macaques, M. sylvanus (Deag, 1977), contradicting the generalisations that "hierarchies are tenuous or absent" in wild groups of primates (Rowell, 1974) and that hierarchies are only produced by 'stress'. These monkeys were also wholly dependent on their

natural resources. Secondly, both dominant and subordinate animals could be shown to play a part in maintaining a hierarchy not too distant from the original concepts of a 'peck-order'. 'Subordinates' avoided animals that on this or other occasions threatened them and 'dominants' threatened animals that on this or other occasions avoided them (Deag, 1977). The two roles were hopelessly dependent on one another. Thirdly, in the case of males, because success in copulating during a short observation period bears an unknown relationship to fertilization success and to a lifetime's production of offspring, poor correlations between rank and access to females, mounting, and so on, are difficult to interpret, and do not necessarily weaken a relationship between rank and reproductive success. With females the case is much stronger. In two studies of free-ranging monkeys, rhesus macaques, M. mulatta (Drickamer, 1974) and gelada baboons, Theropithecus aethiops (Dunbar and Dunbar, 1977), high-ranking females produced more offspring than low-ranking females. With the geladas, this differential was related to the harrassment of subordinates by dominants, thereby causing subordinates to undergo more oestrous cycles before conception than dominants.

5.1b Dominant-subordinate relationships and reproductive success amongst groups of marmosets and their close relatives, the tamarins

Unfortunately, groups of marmoset and tamarin monkeys have been observed mainly in captivity. However, their small size has permitted the maintenance of groups whose social structure closely resembles that of free-living groups - an adult male and female, their immediate offspring and older related (and/or unrelated) animals (Dawson, 1976; Neyman, 1978; Stevenson, in press,a). Probably as a

result, confinement has had less detrimental effect on their behaviour (Trollope, 1977) than that observed with larger primates such as baboons and rhesus monkeys (Rowell, 1967, 1972a).

Hierarchies in captive marmoset groups differ in two major ways from those normally observed in monkeys such as macaques or baboons. In the latter monkeys, hierarchies are reasonably linear (i.e. animal 1 is dominant to animal 2, which is dominant to animal 3, and so on, regardless of sex), but there can be idiosyncrasies to this pattern (Hausfater, 1975; Deag, 1977). The large adult males also predominate at the top of the hierarchy. In some Old World species, females occupy the top ranks, (e.g. talapoin monkeys, Miopithecus talapoin, Dixson et al, 1975). In common marmosets, a male and a female dominate in each group (Epple, 1967, 1975; Rothe, 1975). One male dominates all other males in the group and one female dominates all the other females. There is usually little aggression between the sexes, and Epple (1975) and Rothe (1975) have concluded that the sexes form separate hierarchies. Epple (1975) found no hierarchy among the submissive males or females, whereas from quantitative measurements, Rothe (1975; unpublished work) found two strictly linear rank orders. In family groups, the parents hold the unchallenged alpha positions, whereas the oldest offspring have to defend their beta position against the young of later births (Rothe, cited by Epple, 1975). However, Epple (1975) has found no obvious rank order in her family groups. In wild groups of Geoffroy's tamarin, Saguinus geoffroyi (Dawson, 1976) and the cotton-topped tamarin, Saguinus oedipus (Neyman, 1978), and in captive groups of the saddle-backed tamarin, Saguinus fuscicollis (Epple, 1975), a similar hierarchical system exists. In the pygmy marmoset, Cebuella pygmaea, a female

usually dominates the group (Christen, cited by Epple, 1975). The differences in the hierarchical structure between the marmosets and tamarins, and macaques and baboons are probably related to the lack of sexual dimorphism and the monogamous social groups found among the former monkeys in contrast to the high degree of sexual dimorphism and polygamy found among the latter.

Moreover, in captive and wild groups of marmosets and tamarins, only the dominant female breeds (Epple, 1967, 1970, 1975; Rothe, 1975; Dawson, 1976, 1978; Neyman, 1978; Stevenson, in press, a). Submissive females, although they mate in the presence of a dominant female, only produce offspring after they or the dominant female are removed from the group (Epple, 1967; Rothe, 1975). This is, again, in contrast to other primates where the effects of dominance on female fertility are only partly inhibitory (Rowell, 1970; Drickamer, 1974; Dunbar and Dunbar, 1977). There is no information regarding the breeding success of subordinate male marmosets and tamarins, because dominant males do not completely exclude them from mating with the dominant female (Rothe, 1975).

Maturing female marmosets and tamarins are therefore faced with the prospect of effective reproductive sterility in their natal groups. Little is known about how females solve this problem, as no information is available on how new breeding groups form in the wild. But, female tamarins are known to transfer between groups (Dawson, 1976; Neyman, 1978). Hence, in this chapter, I propose to investigate what social restraints are placed on marmoset sexual development and, in particular, the mechanism(s) by which social dominance inhibits reproduction in females. To this end, careful quantitative behavioural measurements were made in families and peer groups to assess their rank

order and to determine whether or not marmosets form strictly linear hierarchies within each sex. The effects of rank order on reproductive success were then analysed in terms of the monogamous social group structure.

5.2 General procedure

5.2a Structure of the social groups

Families

All consisted of an adult pair and two sets of twin offspring. No offspring were removed to peer groups until after these observations were completed.

Peer groups

Each usually comprised 3 males and 3 females. However, five of the groups comprised 7 animals: three with 4 males and 3 females and two with 3 males and 4 females. All the animals were colony-bred and were removed from their families between 300-550 days of age.

5.2b Observation schedules

Families

Six of the 17 families were observed for a total of 90 hours, as described in Chapter 2. The observations took place when the youngest offspring were 40, 80, and 150 days of age.

Peer groups

26 of the 33 groups were observed for a total of 106 hours (Chapter 2). Aggressive behaviour was rarely seen after the first 2-3 days, and so ten of the groups were observed for 2h. after the animals were placed together, for 2h. on the next day and for one hour on the third day. The remaining 16 groups were observed for 1-2h. after the animals were placed together and for $\frac{1}{2}$-1h. on the next day. Four groups were observed for 2-6h. three months later, to assess the consistency of the original observations.

5.2c Definition of dominant-subordinate interactions

Dominant-subordinate interactions were recorded either as abbreviated notes (peer groups: days 1-3) or by using the computerised system (families; peer groups: third month) (Chapter 2). Dominant or subordinate status was assigned on the basis of interactions between pairs of animals, as interactions involving 3 or more animals were rare. Any interactions involving food or water were excluded, as were squabbles over bits of bark, twigs, etc. The subordinate animal was identified from the following behaviour fully described in Chapter 2:

facial grimace

submissive vocalisations

squealing

flattened ear tufts

fleeing the vicinity of another animal (about 30cm.).

The dominant animal was identified from behaviour associated with aggression:

vocal threat

ear-tuft flicking

genital present

attack (biting and clawing the opponent)

grasp and bite.

Dominant animals did not always respond aggressively, but they were easily identified from the direction in which the subordinate animal displayed its submissive behaviour (usually up to a maximum of 1m.). The dominant animal was given a score of '1' against each animal it dominated for every dominant-subordinate interaction in which it was identified, and a single interaction could involve all the behavioural responses listed. Animals were not given any score for exhibiting

aggressive behaviour if their opponent did not submit. An interaction was only considered as having terminated when the dominant animal started to interact with another animal in the group, when both animals lost sight of each other and the pursuit stopped, or when both animals ceased directing the particular behavioural responses involved at each other.

5.2d Arrangement of dominant-subordinate interactions into a hierarchy

A maitrix was constructed for each group from the dominance scores of each individual. The sequence of individuals in the maitrix was shuffled to discover the order for which the sum of the scores below the diagonal line (top left to bottom right) was minimal and yet placed each individual as high as possible (for an example see Table 5.1). This procedure determined the basic rank of every animal and the hierarchy (or rank order) in each group. In peer groups, providing there were no reversals of assigned status, animals were ranked as dominant to others if they were scored as dominant on 3 or more separate occasions. If there were reversals of status, the prevailing rank at the end of the observations was taken, as this could be decided by only one decisive fight followed by very few interactions. In families, because there were no reversals of assigned status and very few interactions, animals were ranked as dominant to others if they were ever scored as dominant.

On completion of observing each group over the first two or three days, the number of dominant-subordinate interactions between males, between females and between male and female were calculated and compared with what was expected from the overall total (chi-squared test; 2df; Siegel, 1956). This assumed that interactions were

independent of one another. If this test revealed a significant deviation from what was expected ($p < 0.05$), each of the three categories was tested against the others in an attempt to tease out the bias in one or more of the categories (chi-squared test; 2 x 2 table; Siegel, 1956).

The animals were also ranked for age, body weight and ear tuft colour (a dye technique used for identification purposes; see Chapter 2). Correlations were then attempted between these rankings and the animals' basic rank using Kendall's coefficient of concordance (Siegel, 1956).

5.2e Description of pair-bonding behaviour in peer groups

A 'pair bond' was initiated between the dominant male and female within a few hours and was expressed by the relatively high frequencies of the following behaviour (fully described in Chapter 2):

proximity (remaining within arm's length of each other; $\leq$ 10cm.)

follow

huddle (huddling together)

social grooming (grooming one another)

courtship (tonguing, lipsmacking, nuzzling, licking and rubbing each other's bodies)

copulation (mounts accompanied by pelvic thrusting).

The frequencies with which these behaviours were displayed by the dominant pair and the next most frequently interacting male-female pair were then compared using the Wilcoxon matched-pairs signed test (one-tailed; Siegel, 1956).

Vaginal washings from every female were examined daily for spermatozoa for at least four weeks after the group was set up or until spermatozoa were discovered (whichever was the longer period; see Chapter 4).

5.2f Testing the ejacultory response of male offspring and subordinate males in peer groups

Five male offspring (300-510 days of age) from separate family groups and four subordinate males from four different peer groups were tested singly in the observation room with an unfamiliar female. The peer group males had been subordinate for at least 3 months. Each test lasted for 15 min. and vaginal washings were taken from the female before and afterwards. Only females with initially sperm-free lavages were used.

5.2g Blood sampling and hormone assays

Males and females were bled for varying lengths of time before and after their inclusion into a peer group. Females were bled at 2-3 day intervals and males at weekly intervals, and the plasma samples were assayed for progesterone, prolactin and cortisol in females and for only testosterone in males. The frequency of female sampling was sufficient to cover the ovarian cycle of 16.4 $\pm$ 1.7 days (Hearn and Lunn, 1975). The sampling and assay procedures are fully presented in Chapter 2.

5.2h Pregnancy detection

As described in Chapter 4, pregnancies were monitored at least monthly either by radioimmunoassay of plasma progesterone concentrations or by transabdominal uterine palpation.

5.3 Results

5.3a Dominant-subordinate relationships among members of a family

Dominant-subordinate interactions were rare in family groups. Table 5.1 illustrates the ranking of dominance scores in two of the

TABLE 5.1: Dominance scores, determined from the number of interactions between dominant and subordinate animals, of 12 marmosets in two family groups (a) Family 1, and (b) Family 3.

(a)

Dominant animal	Subordinate animal					
	Aσ♂	A♀	Jσ♂$_1$	Jσ♂$_2$	I♀$_1$	I♀$_2$
Aσ♂		0	0	1	6	10
A♀	0		2	3	7	9
Jσ♂$_1$	0	0		2	4	3
Jσ♂$_2$	0	0	0		3	1
I♀$_1$	0	0	0	0		0
I♀$_2$	0	0	0	0	0	

(b)

Dominant animal	Subordinate animal					
	A♀	Aσ♂	Jσ♂	J♀	I♀$_1$	I♀$_2$
A♀		5	2	2	13	15
Aσ♂	0		0	0	9	6
Jσ♂	0	0		0	19	16
J♀	0	0	0		14	14
I♀$_1$	0	0	0	0		0
I♀$_2$	0	0	0	0	0	

A = adult; J = older set of offspring; I = younger set of offspring

six families and the especially small number of interactions between older animals. There were no reversals of assigned status.

The only obvious rank order was that between the youngest offspring and the rest of the family. This was usually caused by the chastisement of the youngsters (grasp bite (inhibited?), hitting and pushing) for attempting to climb on to one of the older animals or persistently huddling too closely to the others, wriggling between them and disturbing their positions. Otherwise, either both the adults or just one adult dominated the family, with the older offspring varying from apparent equality with the parents in some families to subordinate status in others (Figure 5.1). Squabbles between the parents erupted from the female rejecting her partner's mounts or attempted mounts. The rest normally resulted from animals wriggling between huddling animals and disturbing their positions. In half of the families, one of the older offspring appeared dominant to the other (Table 5.1, Figure 5.1). There was no rank order between the youngest animals.

However, the latter situation among the youngest offspring does not last long because young twins normally fight each other between 6-14 months of age and establish a dominant-subordinate relationship. In five families other than those reported here, there were three instances of females attacking and badly injuring their twin sisters, and two instances of males behaving similarly towards their twin sisters. In one of the former cases, the sustained attacks necessitated removal of the injured animal from the family. There was no indication whether or not twin-fighting established a lasting dominant-subordinate relationship.

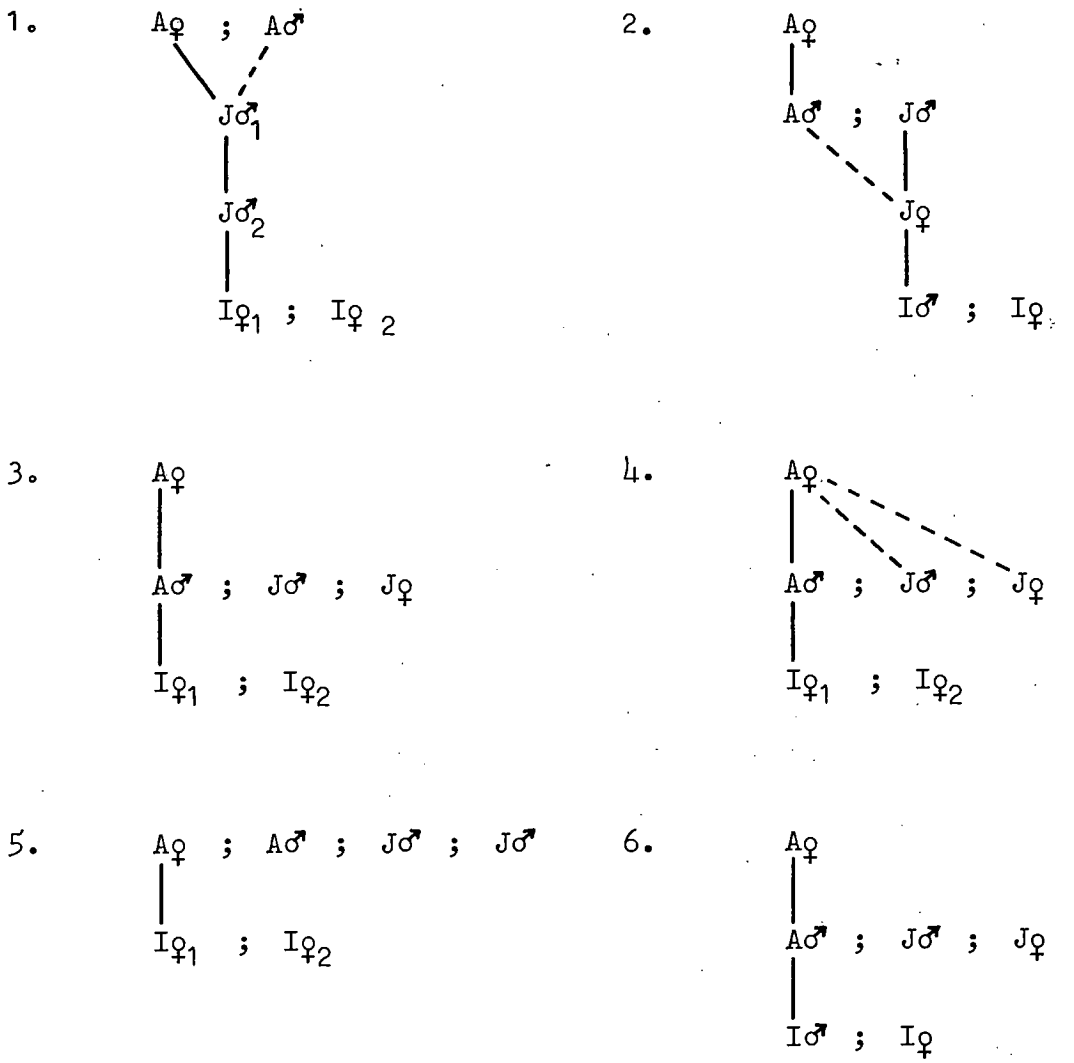


FIGURE 5.1: Diagrammatic representation of the rank order in six marmoset families.

A = adult; J = older set of offspring; I = younger set of offspring

----- = no dominant-subordinate interactions observed (only shown for animals immediately above and below each other).

____ = linking a dominant animal to its subordinate below.

; = linking animals of approximately equal status; see text.

5.3b Reproductive success in families

In family groups only the parents reproduced. However, little sexual behaviour was observed among the offspring or between parents and offspring (see Chapter 4). In six separate families, daughters over 400 days of age have ovulated, as determined by their high plasma progesterone concentrations (> 40 ng/ml). Five male offspring between 300 and 510 days of age ejaculated normal motile spermatozoa when tested with unfamiliar females outwith the family.

5.3c Dominant-subordinate relationships in newly-established peer groups

In newly-formed peer groups, dominant-subordinate interactions were very common, in contrast to the situation in families. A dominant male (Male 1) and female (Female 1) were identified in every group.

In an example from one peer group, observed for the first 3 days after formation, a clear ranking order was achieved among the three males (Table 5.2). Male 2 started the aggressive behaviour in the group, and the reversals of assigned status occurred during the very first interactions between Males 1 and 2. In the peer groups, Males 2 or 3 were usually the first to exhibit aggressive behaviour (60% of the time in groups with 3 males and 3 females). A similar rank order was found among the females (Table 5.2). When all six animals were considered together, intra-sexual rank was not necessarily related to inter-sexual rank and no clear ranking order from 1 to 6 could be achieved. The dominant male and female ranked as dominant to all the rest, but about equal to each other, scoring 1 and 3 respectively. Male 2 and Female 2 were ranked as subordinate to the dominant pair, but equal to each other, scoring 4 each. Female 3 was subordinate to all but Male 3. This latter animal never dominated

TABLE 5.2: Dominance scores, determined from the numbers of interactions between dominant and subordinate animals, of 6 marmosets in peer group 1.

	Subordinate animal					
Dominant animal	Female 1	Male 1	Male 2	Female 2	Female 3	Male 3
Female 1		3	23	38	63	40
Male 1	1		16	7	13	15
Male 2	0	5		4	15	35
Female 2	0	2	4		9	8
Female 3	0	0	6	3		9
Male 3	0	0	0	0	0	

The scores below the diagonal line (6.4%) indicate reversals of assigned status.

any animal in the group. The rank order of this peer group is diagrammatically represented in Figure 5.2.

However, all peer groups were not identical to No. 1. Table 5.3 and Figure 5.3 illustrate the ranking order in peer group 2. It was similar to peer group 1, but among the males, while Male 1 was dominant to Male 2 and Male 2 was dominant to Male 3, Male 1 did not score sufficiently to be clearly dominant over Male 3. However, from the submissive behaviour Male 3 displayed to other animals, it was highly likely that Male 1 was dominant to Male 3. Among the three females, a clear ranking order was achieved. Female 2 started the aggressive behaviour in the group, and her four reversals of assigned status occurred during her very first interactions with Female 1 and Male 1. Females 2 or 3 did not usually aggress first (only 10% of the time). When all six animals were considered together, the rank order was again less clear than when the sexes were treated separately. Male 1 and Female 1 ranked above everyone else, but were approximately equal to each other scoring 4 and 1 respectively (Table 5.3, Figure 5.3). Female 2 ranked well above all but the dominant pair. The remaining three formed a linear order from Female 3 down to Male 3, but they rarely interacted with each other resulting in Female 3 not clearly dominating the most subordinate male.

Peer group 3 illustrated another variation (Table 5.4, Figure 5.4). This was one of the groups only observed for 2 days after formation, but the results from this shorter observation period were not basically different to those obtained from the 3-day period. A clear ranking order was achieved among the males, but among the females, only the dominant animal was clearly identified. Female 2 was only scored as dominant to Female 3 in four out of the six

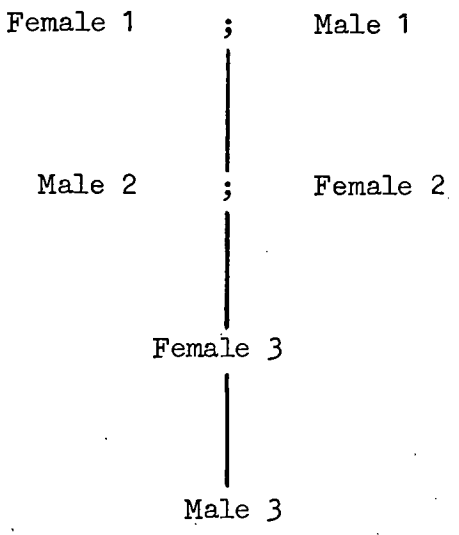


FIGURE 5.2: Diagrammatic representation of the rank order in peer group 1 (Table 5.2)

; = linking animals of approximately equal status; see text.

—— = linking a dominant animal to its subordinate, below.

TABLE 5.3: Dominance scores, determined from the numbers of interactions between dominant and subordinate animals, of 6 marmosets in peer group 2.

	Subordinate animal					
Dominant animal	Male 1	Female 1	Female 2	Female 3	Male 2	Male 3
Male 1		4	63	18	107	2
Female 1	1		32	5	11	15
Female 2	2	2		119	5	20
Female 3	0	0	0		3	2
Male 2	0	0	0	0		3
Male 3	0	0	0	0	0	

The scores below the diagonal line (1.2%) indicate reversals of assigned status.

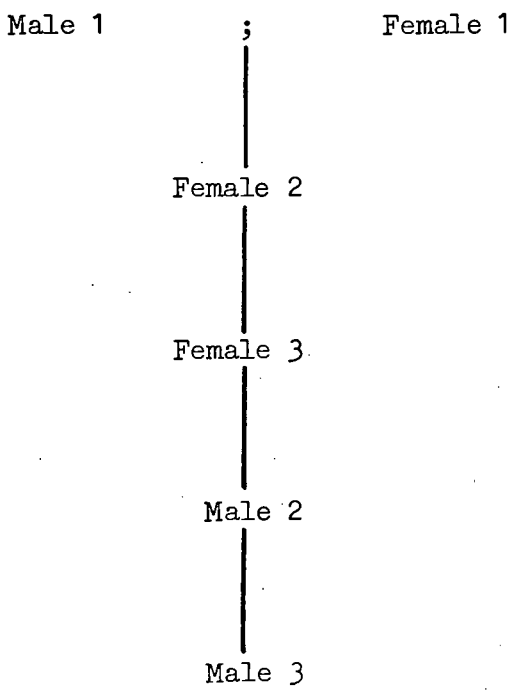


FIGURE 5.3: Diagrammatic representation of the rank order in peer group 2 (Table 5.3)

; = linking animals of approximately equal status

_____ = linking a dominant animal to its subordinate below

TABLE 5.4: Dominance scores, determined from the numbers of interactions between dominant and subordinate animals, of 6 marmosets in peer group 3.

	Subordinate animal					
Dominant animal	Male 1	Female 1	Male 2	Male 3	Female 2	Female 3
Male 1		4	14	10	0	2
Female 1	3		15	29	19	30
Male 2	0	0		30	12	5
Male 3	2+	0	0		1	0
Female 2	0	0	0	0		4
Female 3	0	0	0	0	2	

The scores below the diagonal line (2.8%) indicate reversals of assigned status.

+Only during the very first interactions between Males 1 and 3.

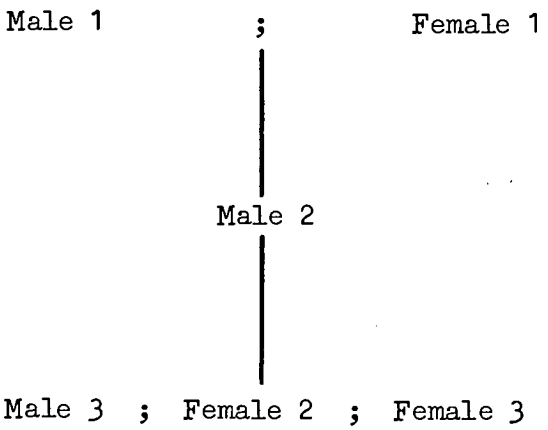


FIGURE 5.4: Diagrammatic representation of the rank order in peer group 3.

; = links animals with approximately equal rank

——— = links a dominant animal to its subordinate below

interactions, and no clear ranking between these two was achieved. When all six were considered, the dominant male and female ranked similarly to groups 1 and 2. Male 2 was ranked as subordinate to the dominant pair, but dominant to all the rest. The rank order between Male 3 and Females 2 and 3 was not clear, mainly because they spent most of their time avoiding the dominant animals. (Such indecisive dominance scores were also found in groups observed for the longer period.) There was no interaction between the dominant male and Female 2, but from her submissive behaviour towards the other dominant animals it was highly unlikely that she would be dominant to the dominant male.

Five of the 26 peer groups contained 7 animals: three comprised 4 males and 3 females and two comprised 4 females and 3 males. An example of the rank order in both types of group is given in Figure 5.5.

In three of the remaining 21 groups, one of the dominant pair dominated its partner as well as the rest of the group. In two, Female 1 dominated the group (e.g. Table 5.5 and Figure 5.6) and in one, Male 1 predominated (Table 5.6, Figure 5.7), illustrating the variety in ranking order from one peer group to the next. Nevertheless, the consistent pattern in 23 out of the 26 peer groups showed the dominant pair holding approximately equal rank to each other and dominating the rest of the group. The ranking order among their subordinates, however, was not so predictable.

5.3d The contribution of each sex to the dominant-subordinate relationships in newly-established peer groups

Of the 21 peer groups with equal numbers of males and females, only four showed the expected distribution of dominant-subordinate interactions between male-male, male-female and female-female interactions (chi-squared test; 2df; $p < 0.99$ to $p > 0.05$). The remaining

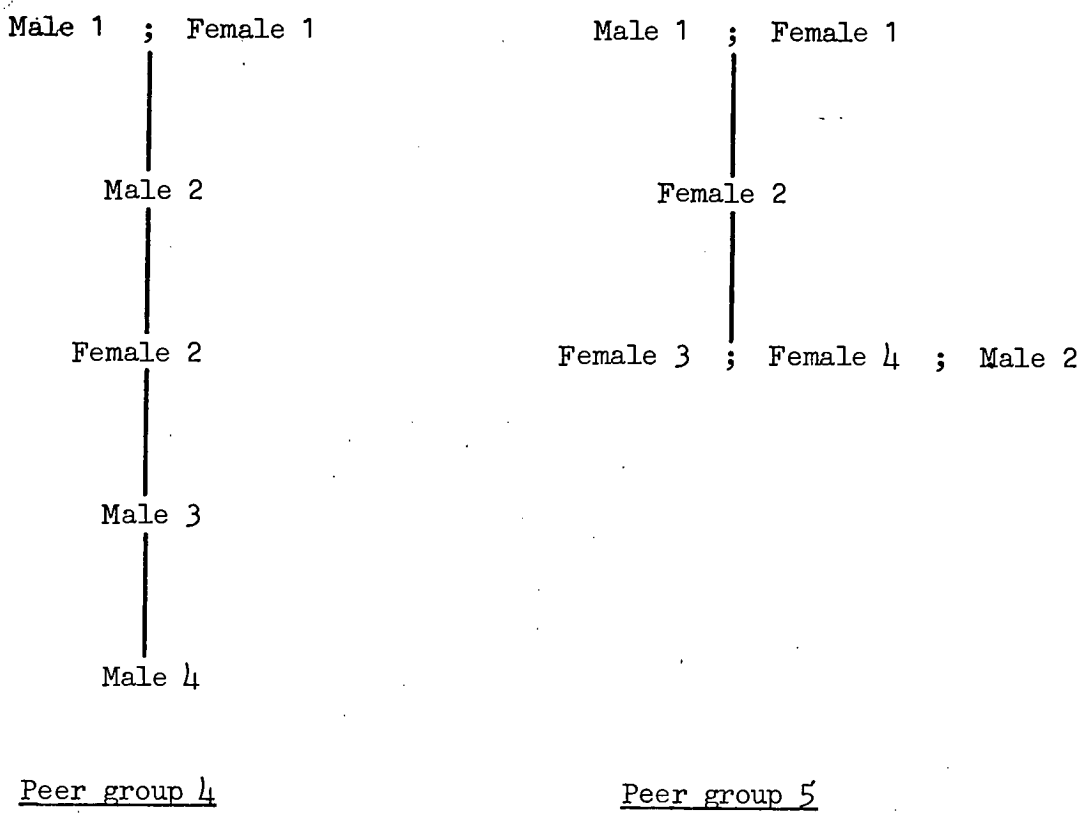


FIGURE 5.5: Diagrammatic representation of the rank order in two peer groups of 7 animals

; = links animals of equivalent rank (regardless of sex)

—— = links a dominant animal to its subordinate below

TABLE 5.5: Dominance scores in peer group 6 as determined by the number of interactions between dominant and subordinate animals.

	Subordinate animal					
Dominant animal	Female 1	Male 1	Female 2	Male 2	Male 3	Female 3
Female 1		18	67	26	31	37
Male 1	1		13	26	25	14
Female 2	0	6		25	16	46
Male 2	1+	2+	5		21	29
Male 3	0	2+	4	0		21
Female 3	0	0	0	1	1	

The scores below the diagonal line (5.2%) indicate reversals of assigned status.

+Only in the very first interactions between these animals.

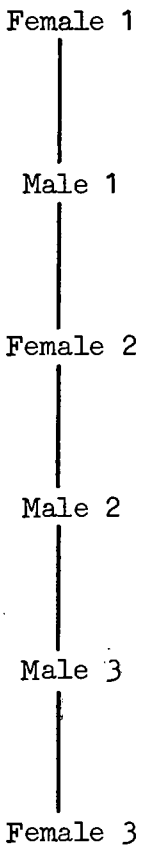


FIGURE 5.6: Diagrammatic representation of the rank order in peer group 6.

——— = linking a dominant animal to its subordinate below

TABLE 5.6: Dominance scores in peer group 7, as determined by the number of interactions between dominant and subordinate animals.

	Subordinate animal					
Dominant animal	Male 1	Female 1	Male 2	Female 2	Male 3	Female 3
Male 1		27	43	31	68	32
Female 1	1		10	35	18	30
Male 2	1	2		4	72	36
Female 2	0	0	3		18	20
Male 3	0	0	0	2		8
Female 3	0	0	0	0	4	

The scores below the diagonal line (2.8%) indicate reversals of assigned status.

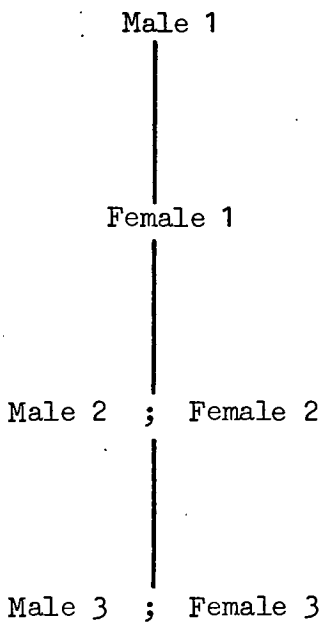


FIGURE 5.7: Diagrammatic representation of the rank order in peer group 7

; = linking animals of approximately equal status

—— = linking a dominant to its subordinate below

17 showed a large difference to the expected distribution ($p < 0.01$ to $p < 0.001$). In four this was due to males interacting significantly more with males than with females (chi-squared test; 1df; $p < 0.001$), in three to females interacting more with females than with males ($p < 0.01$ to $p < 0.001$), and in three to both males and females interacting significantly more with their own sex than with the opposite sex ($p < 0.05$ to $p < 0.001$ for both). In another four groups, males or females did not significantly interact with their own sex more than the opposite sex, but the original significance arose because in two, males squabbled much more among themselves than did females (chi-squared test; 1df; $p < 0.001$) and in the other two, females squabbled more among themselves than did males ($p < 0.05$ to $p < 0.001$). In contrast, the biased distribution of interactions in the remaining three groups was caused by either males or females squabbling far more with the opposite sex rather than with their own ($p < 0.05$ to $p < 0.001$).

Hence, in the majority of peer groups (14 out of 21) dominant-subordinate interactions were inclined to occur amongst animals of the same sex. In the remaining seven groups, dominant-subordinate interactions were prone to occur between animals of opposite sex, or the distribution of interactions within and between the sexes was as expected. However, none of this made any difference to the clarity of rank order achieved. Peer group 3 illustrates the rank order from an expected distribution of dominant-subordinate interactions (Table 5.4, Figure 5.4), peer group 8 illustrates the bias of interactions between the sexes (Table 5.7, Figure 5.8), peer group 2 illustrates the tendency of females to squabble with females instead of males (Table 5.3, Figure 5.3), and peer group 7 illustrates the tendency of males and females to squabble among their own sex instead of with the opposite sex (Table 5.6, Figure 5.7). There was no consistent pattern of one sex dominating the other

TABLE 5.7: Dominance scores in peer group 8, as determined by the number of interactions between dominant and subordinate animals.

	Subordinate animal					
Dominant animal	Female 1	Male 1	Male 2	Female 2	Male 3	Female 3
Female 1		3	24	18	7	46
Male 1	0		15	12	10	20
Male 2	0	4+		17	13	51
Female 2	0	0	13		10	12
Male 3	0	1	7+	4		2
Female 3	0	1	12+	4	0	

The scores below the diagonal line (15.0%) indicate reversals of assigned status.

+Only during the very first interactions between these animals.

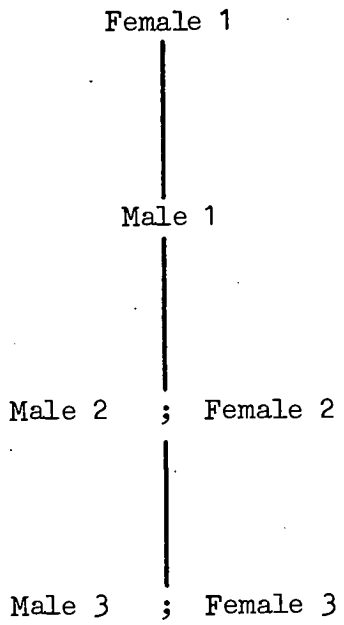


FIGURE 5.8: Diagrammatic representation of rank order in peer group 8.

; = linking animals of approximately equal status

—— = linking a dominant animal with its subordinate below

probably because in two-thirds of the groups, the sexes were prone to interact agonistically among themselves.

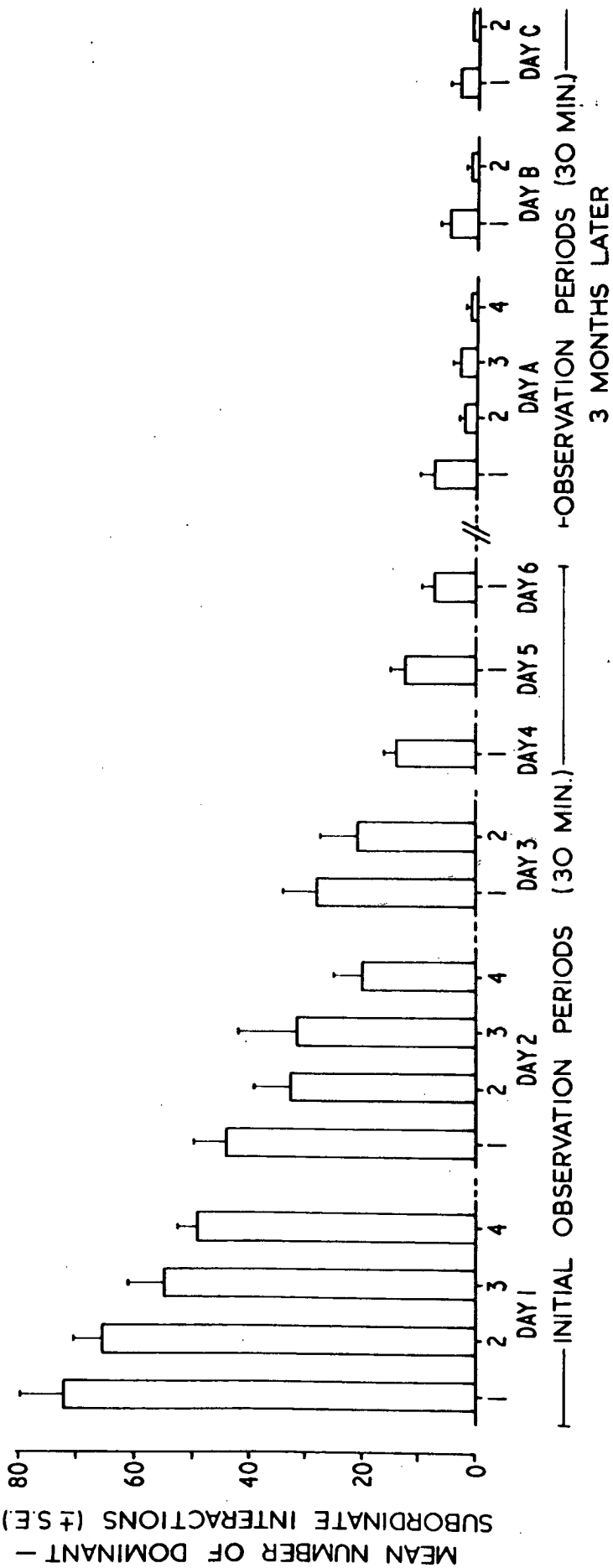
5.3e Reduction of the frequency of dominant-subordinate interactions and the removal of animals from the peer groups

After the first 2-3 days, usually following the removal of one of the subordinate animals because of persistent attacks from one or more dominant animals, the relationships in the peer groups were established and aggressive interactions virtually ceased. By 4-6 days after group formation, the frequency of dominant-subordinate interactions had also dropped to levels comparable with those observed 3 months later (Figure 5.9). Notably the highest number of dominant-subordinate interactions always occurred in the first 30 minutes of observation on each day.

In 76% of the peer groups with equal numbers of males and females (n=21), a subordinate female was removed within 2-3 days of group formation, reflecting the more severe dominant-subordinate interactions among females. 14% of the time a subordinate male was removed. However, in two groups (10%), the dominant female was removed because of her attacks on all but the dominant male. In both these cases, Female 2 took on the dominant role. In the three groups with 4 males and 3 females, two subordinate males were removed from each, and in the remaining two groups with the extra female, two subordinate females were removed from one and a subordinate female and male from the other. No matter whether a group started with 6 or 7 animals, it usually ended up with only 5 after 2-3 days.

Subordinate males seemingly prevented their expulsion from the group by prostrating themselves in front of the dominant male,

FIGURE 5.9: The decrease in the number of dominant-subordinate interactions after the peer groups are formed.



whilst emitting submissive vocalisations and crawling up, huddling against and nuzzling him. Initially dominant males did not tolerate this behaviour, but usually by a day after the group was set up, at least one subordinate male could huddle safely against the dominant male. Such subordinates were never removed from newly-formed peer groups. Unfortunately, this tactic met with little success among females.

5.3f Dominant-subordinate relationships in well-established peer groups

Three months after the groups were set up, dominant-subordinate interactions were rare occurrences (Figure 5.9). The dominant pair (Male 1 and Female 1) could be distinguished by their association (the male following the female, etc.; see Section 5.3h), but no clear rank order between previously dominant and subordinate animals was evident (e.g. peer group 2, Table 5.8).

5.3g Correlations of age, body weight, ear tuft colour and reproductive status with rank order

With males, both age and body weight positively correlated with basic rank (as determined by the dominant-subordinate relationships in each group), indicating that the older and heavier males in each group were likely to dominate the others (Table 5.9). However, no such relationship was found among females. The colour of the dyed ear tufts also bore no relationship to basic rank in either sex.

Reproductive status of the females (i.e. whether or not they exhibited ovarian cyclicity) may have some relationship with their basic rank. In 5 out of 6 groups blood sampled before formation, the ultimately dominant female cycled 1-4 weeks before inclusion into the

TABLE 5.8: Dominance scores in peer group 2 (as determined by the number of interactions between dominant and subordinate animals) three months after group formation.

	Subordinate animal*				
Dominant animal	Male 1	Female 1	Female 2	Male 2	Male 3
Male 1		0	0	0	3
Female 1	0		4	0	0
Female 2	0	0		0	2
Male 2	0	0	0		0
Male 3	0	0	0	0	

There are no scores below the diagonal line indicating no reversals of assigned status.

*Female 3 was removed 2 days after the group was set up.

TABLE 5.9: The relationship between age, body weight and ear tuft colour of male and female marmosets in peer groups and their basic rank.*

Category	Sex	No. of groups	Category rank correlations with basic rank *		
			S	W	p
Age	Male	22	156.5	0.1617	< 0.05
	Female	21	32.7	0.0370	NS
Body weight	Male	17	234.0	0.4048	< 0.01
	Female	19	100.5	0.1392	NS
Ear tuft colour (dyed)	Male	19	8.0	0.0111	NS
	Female	20	78.7	0.0983	NS

* As determined by the number of interactions between dominant and subordinate animals in each group.

W = Kendall coefficient of concordance (Siegel, 1956)

S = sum of squares

p = probability associated with W.

NS = not significant ($p > 0.05$).

group. In two of the five, subordinates did not cycle previously and did not challenge the cyclic female for the top female position. In another two of these cases, another female, apart from the ultimate dominant, also cycled prior to inclusion into the group. The number one position was bitterly disputed between these two, in one instance for three hours. These squabbles were the longest and most severe ever observed in peer groups. Oddly enough, either none of the battling females or just the subsequent loser ovulated at this time. The loser (Female 2) eventually had to be removed within 2-6 days because of the vicious attacks from the dominant female. However, in a similar situation, there was no dispute over rank order between the previously cycling females and Female 2 was not removed (e.g. Figure 5.14c). In the sixth peer group, the dominant female did not cycle immediately before the peer group was set up, but Female 2 did. In this instance there was little dispute over the top position.

5.3h Pair bonding behaviour

Within a few hours of placing each group together, a 'pair bond' was initiated between the dominant male and female, but not between any of the other group members. This pair bond was usually expressed by the two animals frequently remaining within arm's length of each other (proximity), following each other (follow) usually displaying piloerection and an arched-back posture, huddling together (huddle) and exhibiting intimate and unhurried social grooming, courtship and copulatory behaviour, as illustrated by peer groups 2, 3 and 6 (Figures 5.10-5.12). The first three behaviours were significantly associated with the dominant pair, but the latter three were not (Table 5.10). In the case of social grooming, the lack of

FIGURE 5.10: The affiliative behaviour shown by male-female 'pairs' in peer group 2 (Days 1 - 3).

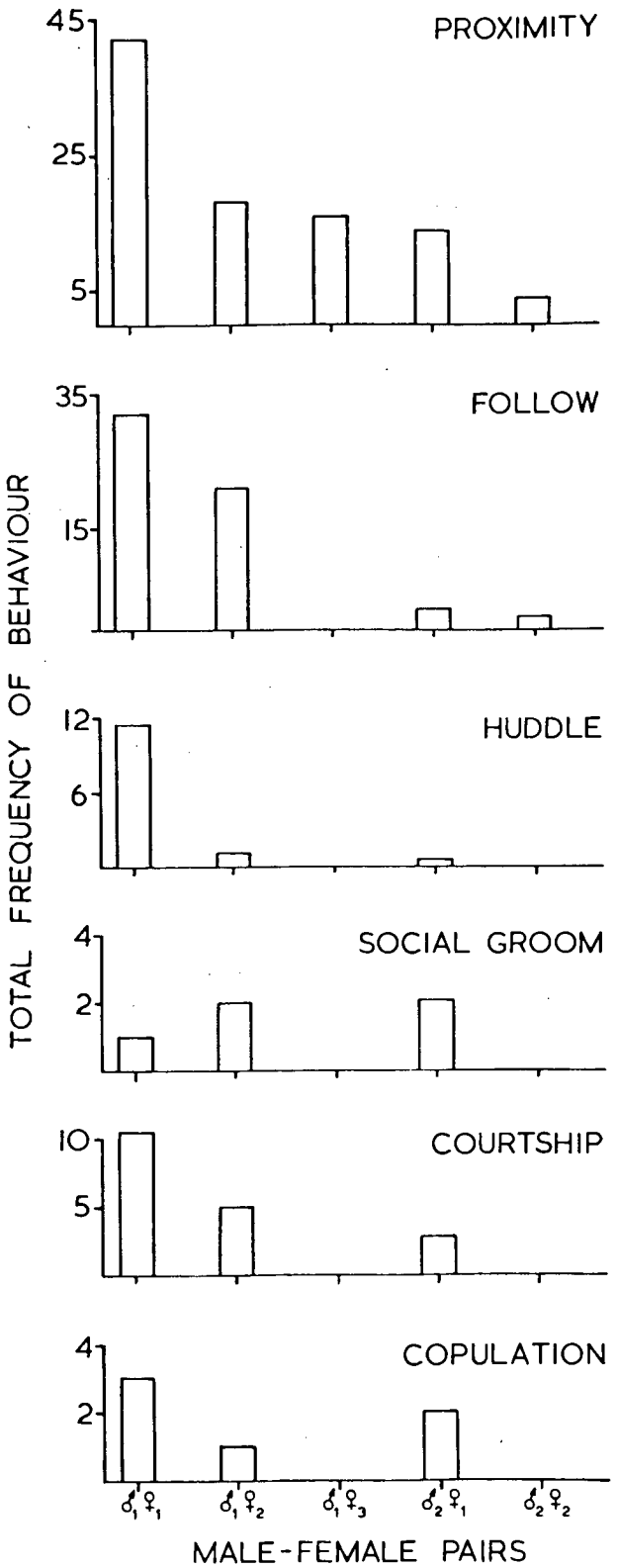


FIGURE 5.11: The affiliative behaviour shown by male-female 'pairs' in peer group 3 (Days 1 - 2).

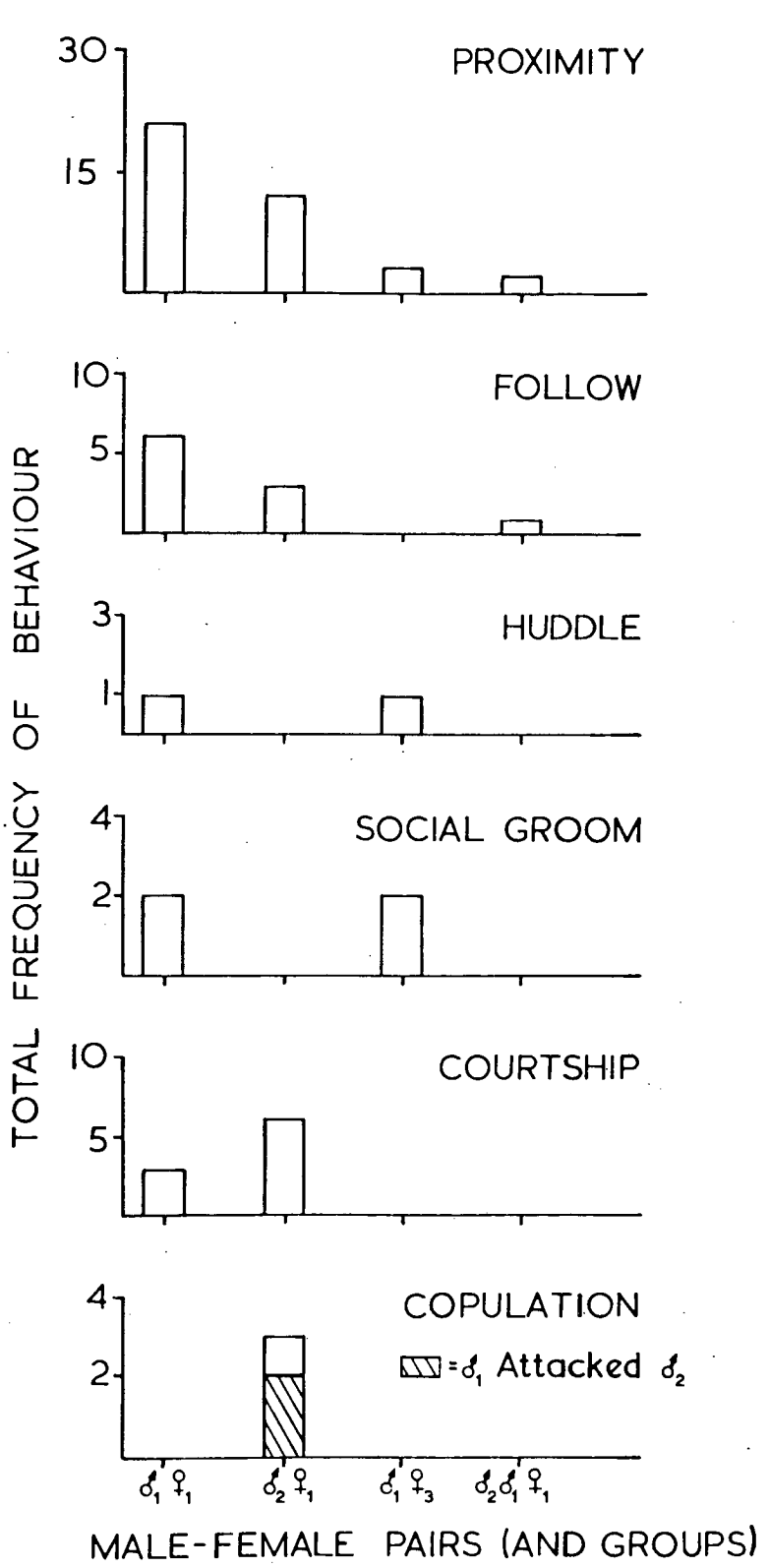


FIGURE 5.12: The affiliative behaviour shown by male-female 'pairs' in peer group 6 (Days 1 - 2).

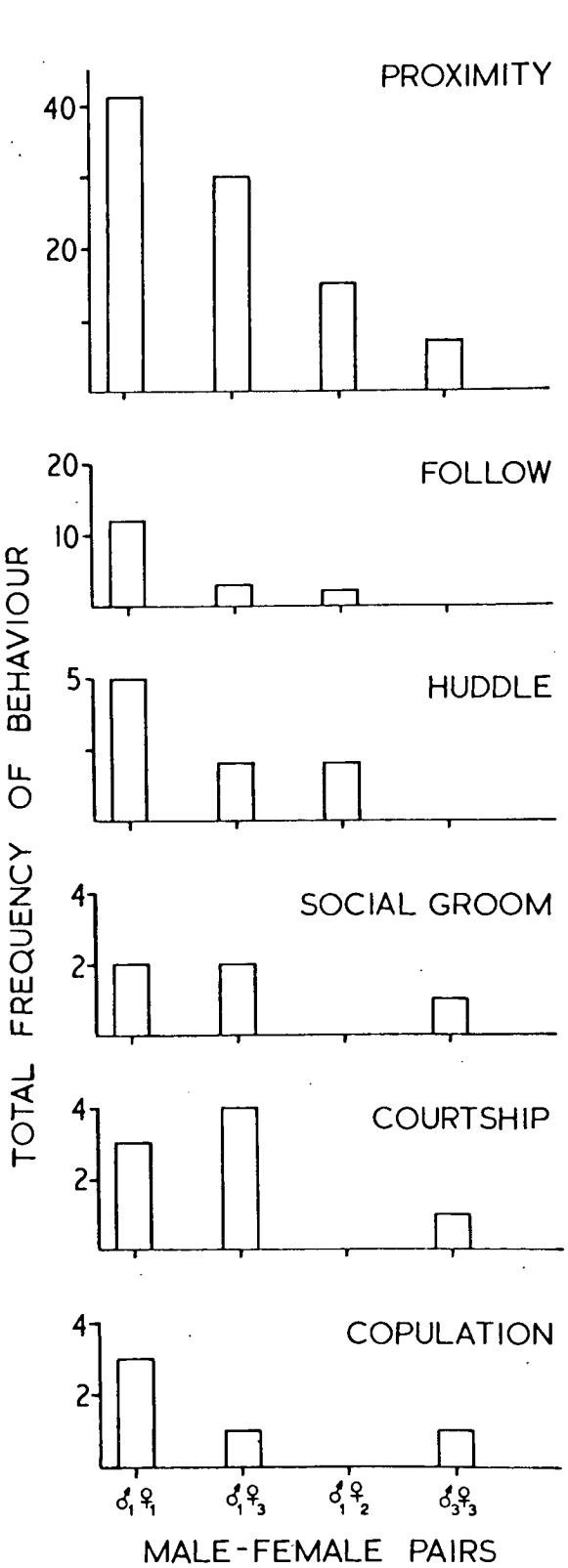


TABLE 5.10: Comparison of the frequencies of social interactions between the dominant male and female with the other most frequently interacting male-female pair in each peer group.

Behaviour	No. of groups	T	p
Huddle	19	0.0	< 0.005
Proximity	19	12.5	< 0.005
Follow	18	31.0	< 0.01
Social groom	10	14.0	NS
Courtship	13	27.5	NS
Copulation	12	25.5	NS

T = sum of the ranks with the less frequent sign (Wilcoxon matched-pairs signed-rank test (one-tailed) Siegel, 1956).

p = probability associated with T.

NS = not significant ($p > 0.05$).

association is probably because subordinate males and females could elicit grooming from either of the dominant pair while submitting to them. With courtship and copulatory behaviour it is because the pair bond did not completely exclude either the dominant male from copulating with other females, or the dominant female from copulating with other males. However, dominant males aggressively disrupted mounting of the dominant female by a subordinate male (4 instances, e.g. Figure 5.11) and on five occasions dominant females dislodged male partners mounting subordinate females. Subordinate males attacked for mounting or attempting to mount the dominant female did not need to be removed from the group, but on 3 occasions subordinate females had to be removed because of injuries inflicted by the dominant female following the subordinate's copulatory behaviour with the dominant male (e.g. Figure 5.12). In the two other instances, the subordinate female was persistently driven out of sight by the dominant female. Nevertheless, in two peer groups the dominant male did not react to a subordinate male mounting the dominant female.

Males were possibly attracted to subordinate females because in some groups (e.g. group 11, Figure 5.14c) these females were cycling before the peer groups were formed. However, by one way or another, the dominant pair always ended up copulating with each other. On some occasions (n=6) this was made easier because while the female initially attractive to the dominant male was not Female 1, she was also extremely submissive to this male and would never let him approach near enough to mount.

Subordinate males also mounted subordinate females and spermatozoa were recovered from the vaginal washings, but these animals did not exhibit the intimate and unhurried licking, tonguing and face-nuzzling behaviour of the copulating dominant pair.

The association of the dominant pair was still evident when peer groups were observed 3 months after they were formed. As peer group 2 illustrates (Figure 5.13), no other male-female pair had developed the affiliative behaviour of the dominant animals.

5.3i The reproductive success and break-up of the peer groups

In every peer group only the dominant female became pregnant. The other females did copulate with the males, but their low plasma progesterone concentrations, over 2 months or more, indicated that they did not ovulate. As Figure 5.14 illustrates, only the dominant female persisted in exhibiting any ovarian cyclicity. However, subordinates might cycle previous to their inclusion in the group and might undergo one cycle immediately following the formation of the group (Figure 5.14c). Such cycles were rather contracted in comparison to those of the dominant females. In this case (Figure 5.14c), the cycling subordinate held the lowest female rank. None of the females in the groups illustrated were found to be pregnant at this time, but the plasma progesterone profile of the dominant female in peer group 11 (Figure 5.14c) suggests that she may have become pregnant and spontaneously aborted at an early stage because of the secondary rise in progesterone at the end of the 'cycle'. Females could be palpated as pregnant from 3-4 weeks of gestation (Hearn and Lunn, 1975).

By the time the peer groups had been established for 3-5 months, most of the dominant females had become pregnant. Their subordinates, however, still showed no sign of ovarian cyclicity (Figure 5.15) and none were palpated as pregnant. When young were born to the dominant female, they were frequently carried and cared for by the dominant and subordinate animals. There were no marked differences in carrying frequencies between subordinate animals

FIGURE 5.13: The affiliative behaviour shown by male-female 'pairs' in peer group 2 three months after formation.

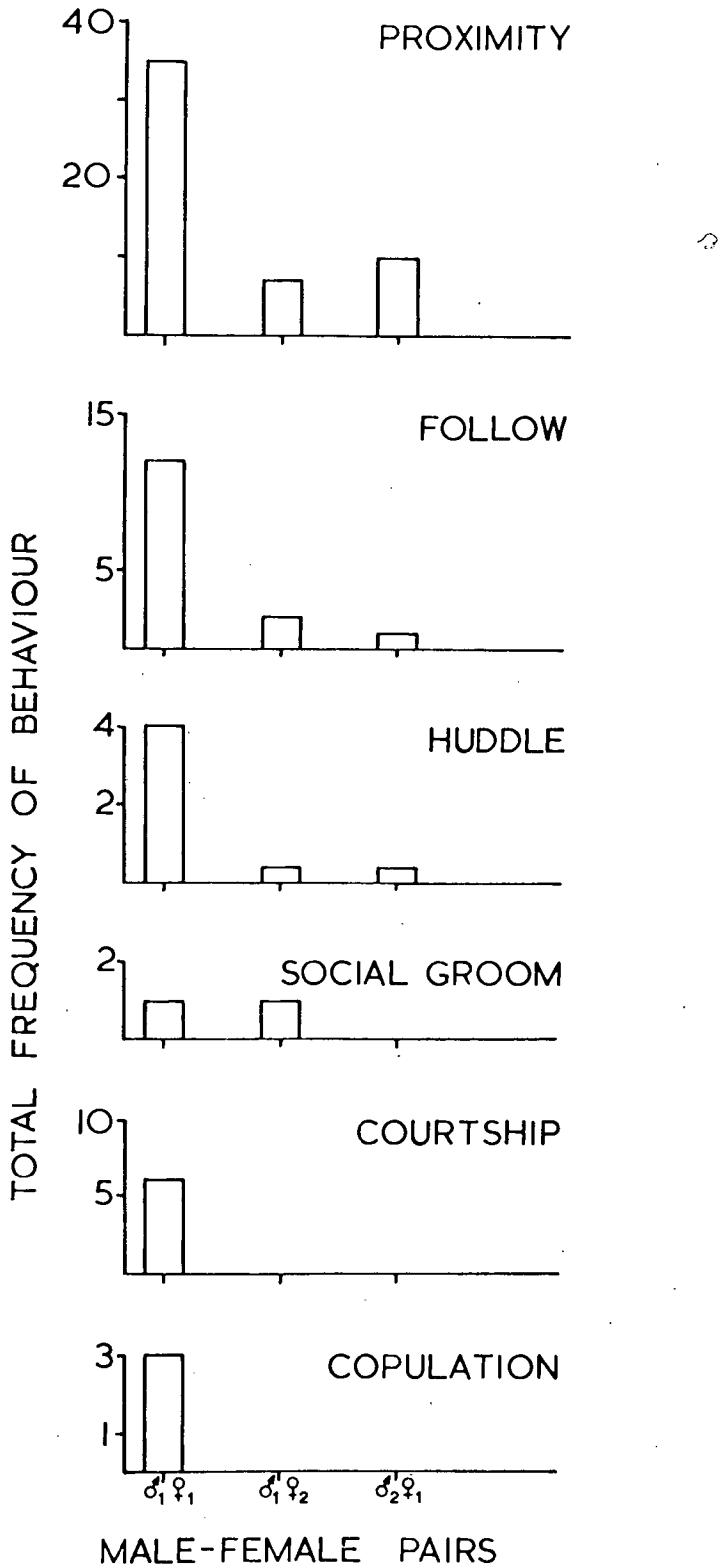


FIGURE 5.14: Plasma progesterone concentrations in female marmosets before and after their inclusion into 3 peer groups, (a) peer group 9, (b) peer group 10 and (c) peer group 11.

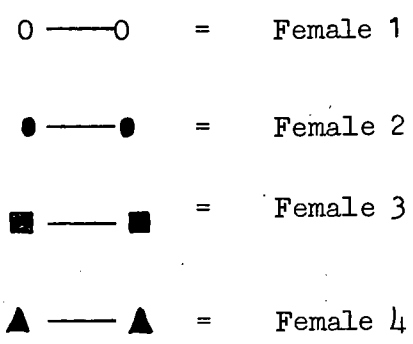
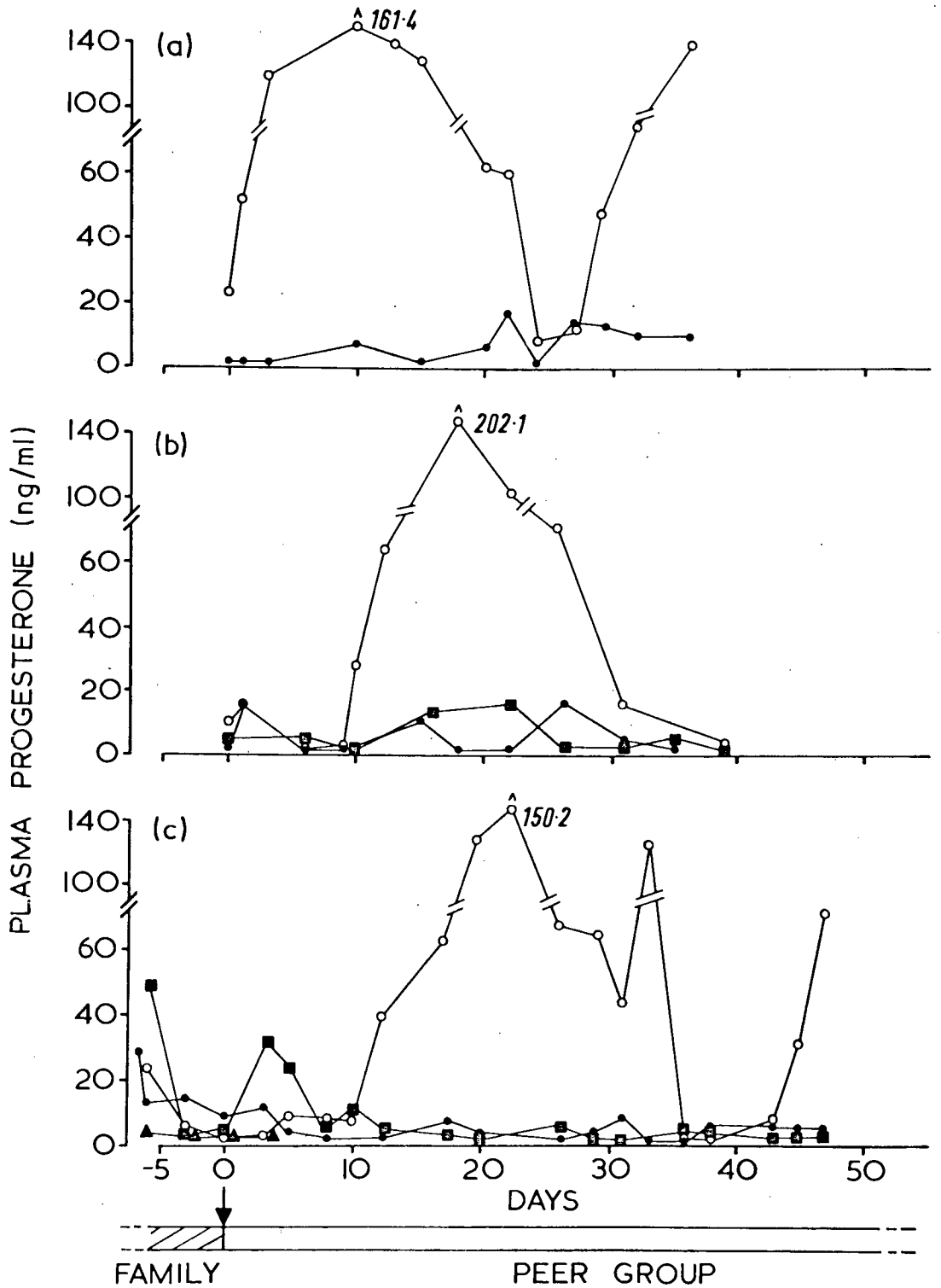


FIGURE 5.14: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140
100
60
40
20
0
(a)
161·4
(b)
202·1
(c)
150·2
-5
0
10
20
30
40
50
DAYS
FAMILY
PEER GROUP

FIGURE 5.15: Plasma progesterone concentrations in female marmosets after 3-5 months in a peer group (a) peer group 12, (b) peer group 13, (c) peer group 20, (d) peer group 14 and (e) peer group 15.

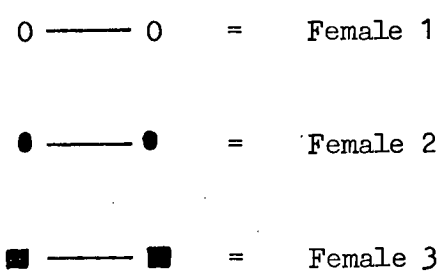
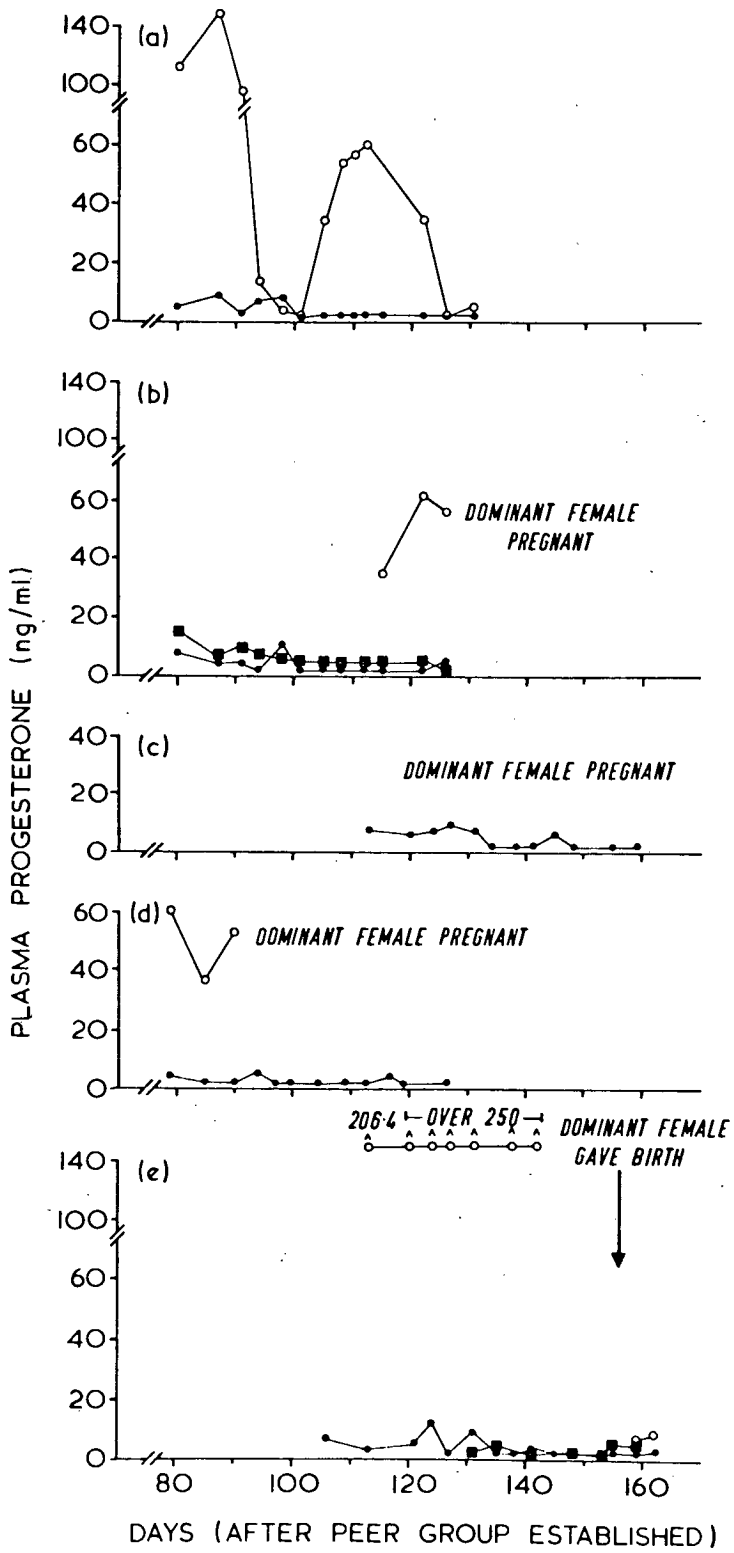


FIGURE 5.15


(a)
(b)
DOMINANT FEMALE PREGNANT
(c)
DOMINANT FEMALE PREGNANT
(d)
DOMINANT FEMALE PREGNANT
206·4
OVER 250
DOMINANT FEMALE GAVE BIRTH
(e)
PLASMA PROGESTERONE (ng/ml)
DAYS (AFTER PEER GROUP ESTABLISHED)

(casual observations of the groups). After the young were weaned, the dominant female attacked one or more of the subordinate females and consequently the peer groups were either disbanded 1 month (n=1) or 2-3 months (n=9) after the young were born. The dominant pair and their young were left in their own cage, while the remaining animals were removed and either separated into male-female pairs, or paired with an experienced adult of the opposite sex. All of the latter pairs copulated and spermatozoa were recovered from the vaginal washings within 2-19 days of pairing (n=14); 9 of the previously subordinate females became pregnant within 10-126 days of pairing, while plasma progesterone concentrations in the remaining 5 indicated that they ovulated within 12-86 days of pairing (e.g. the 2 females in Figure 5.16). Four of the pregnant females and two of the females that ovulated remained in the same room beside their dominant female.

There was little evidence to suggest whether there was suppression of fertility in subordinate males, as the dominant males did not completely exclude others from mating with the dominant females. Subordinate males could still ejaculate spermatozoa when presented with test females outwith the group. These spermatozoa were motile, with apparently normal gross morphology and appeared in similarly large numbers to ejaculates from breeding males. Moreover, subordinate males neither suffered from chronically reduced plasma testosterone concentrations (Table 5.11) nor from attacks from the dominant male following the dominant female giving birth. Nevertheless, subordinate males did lose condition and body weight if they remained in the peer group until the offspring reached 250-400 days of age (n=2).

FIGURE 5.16: Plasma progesterone concentrations in subordinate female marmosets before and after removal from peer group 10.

● ——— ● = Female 2

■ ——— ■ = Female 3

FIGURE 5.16

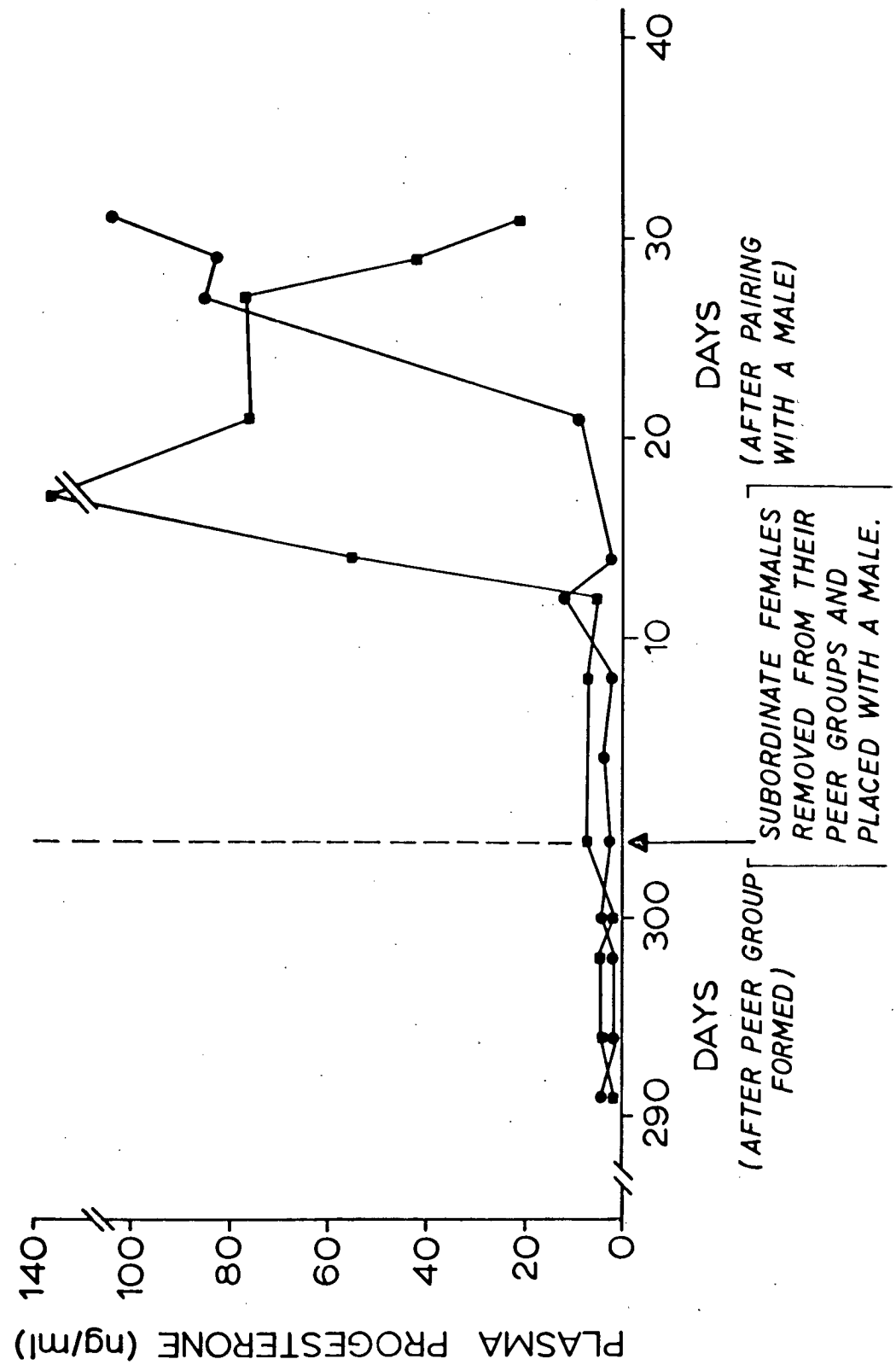

PLASMA PROGESTERONE (ng/ml)
140
100
80
60
40
20
0
290
300
10
20
30
40
DAYS
(AFTER PEER GROUP FORMED)
DAYS
(AFTER PAIRING WITH A MALE)
SUBORDINATE FEMALES REMOVED FROM THEIR PEER GROUPS AND PLACED WITH A MALE.

TABLE 5.11: Plasma testosterone concentrations in peer group males

Peer group	Plasma testosterone (ng/ml)†		
	Male 1	Male 2	Male 3
2	4.2 ± 2.8 (1.1 - 20.8)	2.6 ± 1.6 (1.1 - 12.4)	3.9 ± 1.2 (1.1 - 8.7)
7	6.6 ± 3.5 (1.1 - 17.8)	1.2 ± 0.2 (1.1 - 1.9)	4.0 ± 3.0 (1.1 - 10.2)
12	15.7 ± 5.0 (1.1 - 31.3)	8.4 ± 4.6 (1.1 - 22.4)	1.1 ± 0.2* (1.1 - 1.2)
16	3.9 ± 2.1 (1.1 - 15.2)	5.5 ± 2.2 (1.1 - 14.6)	1.2 ± 0.2 (1.1 - 2.5)
15	23.0 ± 6.6 (3.9 - 50.9)	13.8 ± 5.6 (1.1 - 26.8)	———
17	3.7 ± 2.7 (1.1 - 11.9)	1.2 ± 0.2 (1.1 - 1.8)	———

† Mean ± s.e. (range)

* $p < 0.05$ (Student's t-test against Male 1).

5.4 The relationship between female reproductive inhibition and their plasma prolactin and cortisol levels

Prolactin release in response to specific or non-specific stress has been reported in several species (Raud, Kiddy and Odell, 1971; Horrobin, 1973; Lamming, Moseley and McNeilly, 1975; Friesen et al, 1978; Bowman, Dilley and Keverne, 1978; Keverne, in press). Elevated prolactin levels (hyperprolactinaemia) have also been linked with inhibition of female reproductive function (high-prolactin-amenorrhea) in both humans (Besser, Parke, Edwards, Forsyth and McNeilly, 1972; Tyson, Friesen and Anderson, 1972; van Look, McNeilly, Hunter and Baird, 1977b and Baird et al, in press) and talapoin monkeys, M. talapoin (Bowman et al, 1978). Feasibly, under the stress of attack from the dominant female, subordinate female marmosets may exhibit elevated prolactin levels in association with their anovulatory condition. Increased adrenal activity has also been related to 'stress' and reproductive inhibition in rodents (Brain, 1971), tree shrews, Tupaia belangeri (von Holst, 1969) and several other mammals (Brown, 1975). Accordingly, three peer groups were established to investigate these possibilities along with eleven well-established ones.

5.4a Procedure

The methods were similar to those described in the general procedure (section 5.2). 18 young marmosets were chosen from family groups and randomly assembled into three groups of 6 animals, comprising 3 males and 3 females. Five to six weeks prior to and after inclusion into the peer groups, females were bled at 2-4 day intervals and the samples were analysed for their plasma content of prolactin and progesterone (see Chapter 2). In another eight well-established peer groups (of 3-10 months standing) females were randomly blood sampled

and the plasma analysed for prolactin and progesterone, and in two of these and a further three, females were randomly sampled for cortisol and progesterone measurements (as described in Chapter 2).

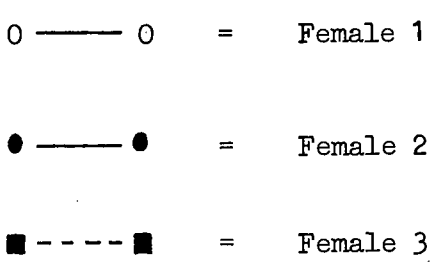
All these animals were bled within 3 min. of removal from their cage. The three peer groups formed were observed for a total of five hours over three days and their rank order was assessed (see section 5.2).

5.4b Results

Prior to their inclusion into peer groups, plasma prolactin concentrations were erratic in all young female marmosets (Figures 5.17 and 5.18). They bore no consistent relationship with their ovarian cyclicity (e.g. Figure 5.17). However, in 4 out of the 8 days in which extremely high prolactin levels were found (over 50 ng/ml), females in at least two different animal rooms were simultaneously affected.

Following the establishment of the three peer groups, plasma prolactin levels remained erratic for about 10-20 days, regardless of status (Figures 5.17 and 5.18). In one peer group, the prolactin levels of the dominant female then remained low while the levels of the remaining subordinate (Female 3) were persistently elevated (Figure 5.17). The dominant female ovulated 25-29 days after inclusion into the peer group, but the subordinate(s) showed no sign of ovarian activity (from their low progesterone levels, $<$ 10ng/ml.; Figure 5.17). Female 2 was removed after 6 days because of violent attacks from the dominant female, but her prolactin was not elevated. However, the picture was not so clear in the other two groups. In both, Female 3 died shortly after experiencing extremely high levels of prolactin ($>$ 50 ng/ml.; Figures 5.18 a and b), despite the lack of attacks from

FIGURE 5.17: Plasma prolactin and progesterone concentrations in three female marmosets before and after their inclusion in peer group 16.



* Female 2 removed from peer group because of attacks from Female 1.

FIGURE 5.17

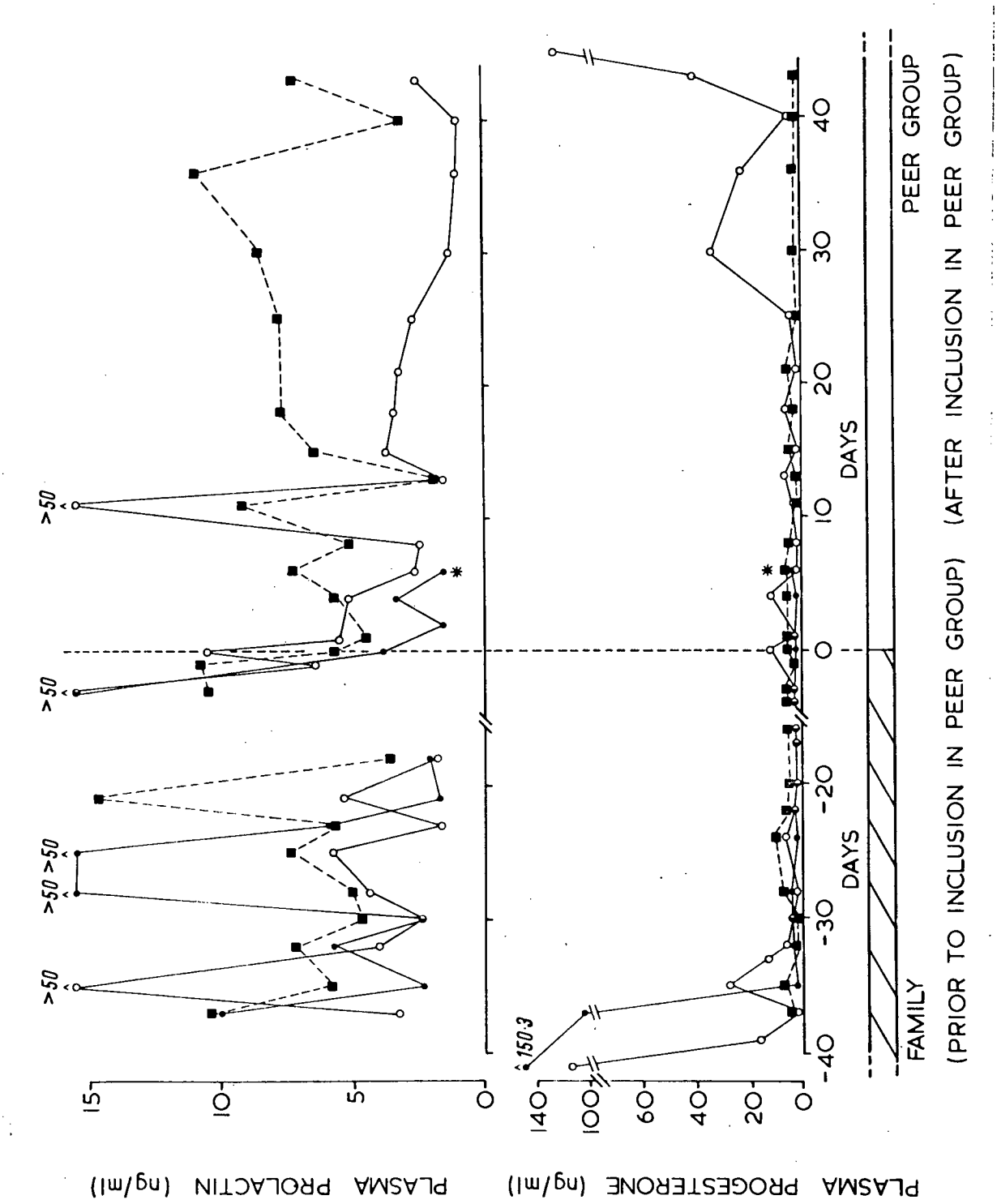
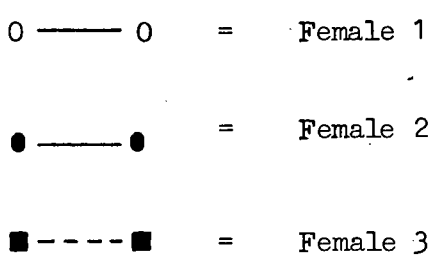
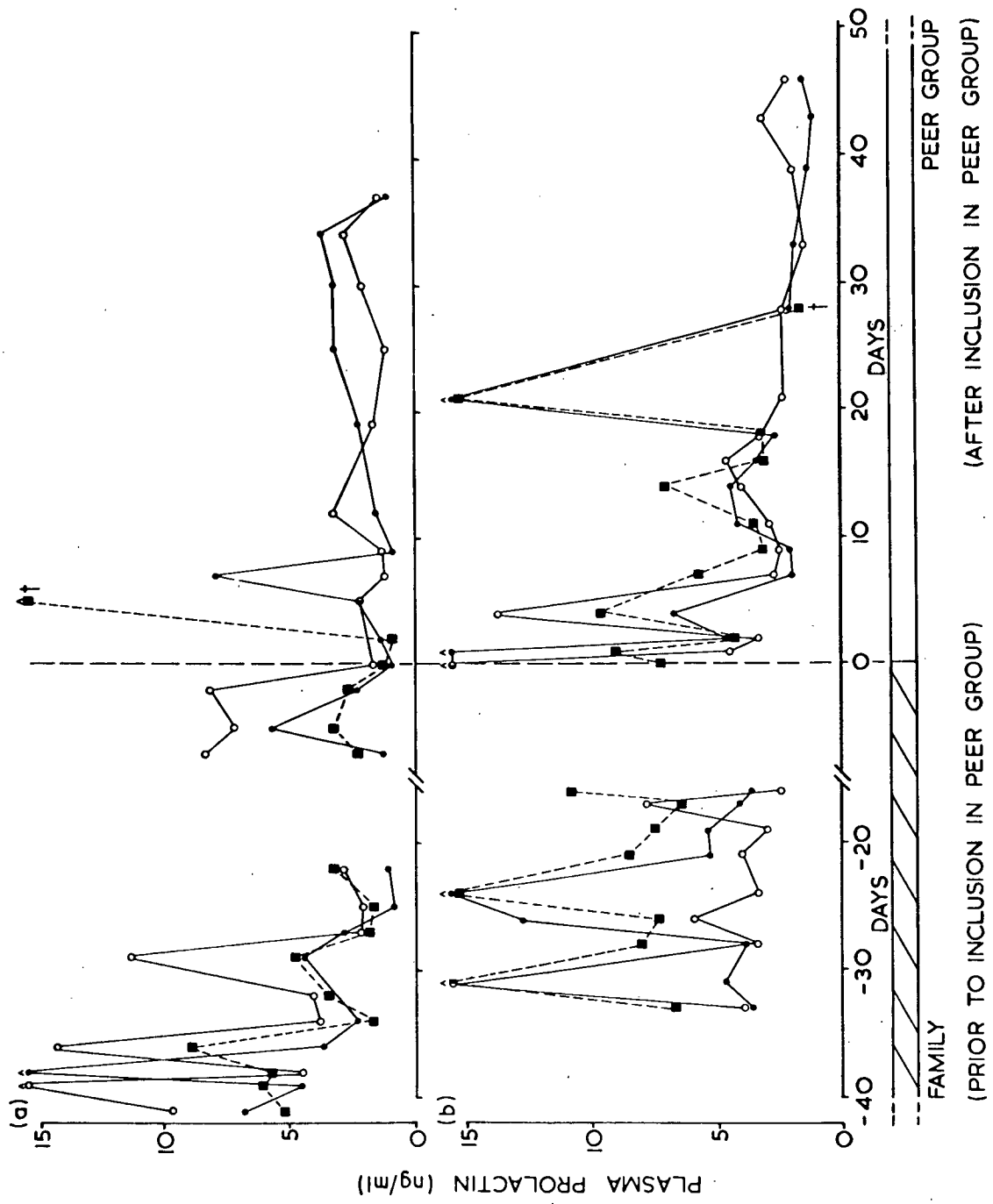

PLASMA PROGESTERONE (ng/ml)
PLASMA PROLACTIN (ng/ml)
DAYS
DAYS
PEER GROUP
FAMILY
(PRIOR TO INCLUSION IN PEER GROUP) (AFTER INCLUSION IN PEER GROUP)
>50
>50
>50
>50
>50
150·3
*
*

FIGURE 5.18: Plasma prolactin concentrations in six female marmosets before and after their inclusion in two peer groups, (a) peer group 7 and (b) peer group 2.



† Female 3 died shortly afterwards.

FIGURE 5.18


PLASMA PROLACTIN (ng/ml)
(a)
(b)
15
10
5
0
-40
-30
-20
10
20
30
40
50
DAYS
FAMILY
PEER GROUP
(PRIOR TO INCLUSION IN PEER GROUP)
(AFTER INCLUSION IN PEER GROUP)

Females 1 and 2 or any visible injury, and despite repeated hand-feeding. There was little difference between the prolactin levels of the remaining females (1 and 2).

Plasma prolactin levels in well-established groups produced no differences between the dominant female and her subordinates, except in 2 out of the 8 groups (Table 5.12). However, 11 out of the 12 subordinates did show high levels of prolactin not shown by dominants. For example, the top values found in the 7 dominants varied from 1.1-6.6 ng/ml., whereas in 11 of the subordinates it varied from 6.9-44.0 ng/ml. (Table 5.12). In contrast, plasma cortisol levels showed no relationship with rank in well-established groups, with both subordinates and dominants having high and low values (Table 5.13).

5.5 Discussion

5.5a Dominant-subordinate relationships in marmoset groups

In families, dominant-subordinate interactions were too infrequent to give a clear indication of the hierarchy. However, the adults, especially the adult female, tended to dominate the others and the youngest offspring always held the lowest ranks (similar to Rothe's (1975) findings). Nevertheless, the youngest twins eventually fought each other around the time of the onset of puberty (approximately 200-250 days of age), but a dominant-subordinate outcome was not always obvious, as found by Poole, Stevenson and Sutcliffe (1978).

In peer groups, initial fighting usually produced a dominant male and female of approximately equal rank (in 23 out of 26 groups). Rank orders within the sexes were strictly linear in some groups (e.g. Table 5.5 and Figure 5.6) and in others, only the dominant animal ranked above the rest (e.g. Table 5.4 and Figure 5.4), reflecting the

TABLE 5.12: Plasma prolactin concentrations of female marmosets in eight well-established peer groups

Peer group	Plasma prolactin (ng/ml)		
	Female 1	Female 2	Female 3
3	1.0 ± 0.1† (0.7 – 1.1)	5.5 ± 1.4* (2.1 - 32.0)	4.4 ± 0.1* (1.2 - 11.2)
5	(pregnant)++	5.1 ± 0.8 (1.9 - 15.0)	-
6	3.6 ± 0.7 (2.6 - 5.6)	3.0 ± 0.4 (1.2 - 4.7)	3.6 ± 1.7 (1.7 - 8.6)
9	4.2 ± 0.9 (2.4 - 6.6)	3.4 ± 0.2 (1.6 - 6.9)	-
10	4.2 ± 0.5 (2.8 - 5.3)	3.3 ± 1.2 (6.6 - 11.5)	6.9 ± 3.7 (1.2 - 25.0)
17	3.3 ± 0.2 (2.7 - 3.8)	6.1 ± 1.9 (2.1 - 44.0)	4.5 ± 1.0 (1.3 - 10.0)
18	4.1 ± 0.4 (3.1 - 4.9)	-	8.5 ± 1.9 (1.8 - 24.0)
19	2.3 ± 0.1 (2.1 - 2.6)	7.0 ± 1.1** (4.7 - 8.3)	

† Mean ± s.e. (range)

++ Prolactin known to be elevated during pregnancy (A. McNeilly, personal communication)

* $p < 0.05$, ** $p < 0.01$ (Student's t-test against Female 1).

TABLE 5.13: Plasma cortisol concentrations from female marmosets in six well-established peer groups

Peer group	Plasma cortisol (µg/100ml)		
	Female 1	Female 2	Female 3
2	184.0 ± 11.6† (160 - 212)	238.1 ± 50.0 (181 - 397)	88.2 ± 12.3 (52 - 106)
4	189.0 ± 8.5 (173 - 207)	182.4 ± 16.6 (158 - 231)	-
5	(pregnant)	240.0 ± 16.6 (189 - 261)	-
6	190 ± 5.6 (178 - 203	280.0 ± 54.8 (163 - 382)	-
7	414.5 ± 76.0 (276 - 614)	285.0 ± 41.5 (213 - 397)	-
10	523.0 ± 68.8 (352 - 689)	-	320.0 ± 36.4 (218 - 394)

† Mean ± s.e. (range)

contrasting results reported by Rothe (1975) and Epple (1967), respectively. However, in antithesis to both these reports, hierarchies between the sexes were also found (e.g. Tables 5.5-5.7 and Figures 5.6-5.8). Furthermore, in 4 of the 21 peer groups with equal numbers of males and females, dominant-subordinate interactions within and between sexes were distributed as expected, and in another 3, males and/or females squabbled significantly more with the opposite sex than with members of their own sex. Nevertheless, in the majority (14 out of 21) males and/or females interacted significantly more with members of their own sex and this explains why intra-sexual rank was not always related to inter-sexual rank. Thus the hierarchy of a marmoset peer group is mainly the product of two separate hierarchies generated by males fighting with males and females fighting with females, as originally proposed by Epple (1967). This explanation reflects the more savage and prolonged fighting between animals of the same sex. In this respect the marmoset is again different to monkeys such as Barbary macaques, M. sylvanus (Deag, 1977), yellow baboons, P. cynocephalus (Hausfater, 1975) and talapoin monkeys, M. talapoin (Dixson, Everitt, Herbert, Rugman and Scruton, 1973) where there is a clearly defined hierarchy between the sexes.

As predicted from other studies of captive primates, aggressive and dominant-subordinate interactions in peer groups had decreased 3-4 days after they were set up, probably because fighting had resulted in the formation of predictable relationships which make up the hierarchies (Rowell, 1972; Deag, 1977), and because, as Epple (1967) had found, a subordinate was removed. However, the removal of animals was not related to limited cage space, as in much larger cages (3.25 x 2.45 x 2.45 m. connected to an outside enclosure of 3.0 x 2.6 x 2.6 m.)

Epple (1967) could never keep more than 2-3 unrelated adults of the same sex in a group. The animal removed from peer groups was not always of the lowest rank (within or between sexes). If there was any major dispute for the top ranking position in either sex, the loser (usually Male or Female 2) might have to be removed instead. This was especially noticeable in the case of females. Furthermore, as the majority of subordinates removed were female, female marmosets seem less tolerant of their nearest rivals than males. Differences between the bases for male and female hierarchies were also underlined by the significant relationship of age and weight with high rank in males but not in females (Table 5.9). This might explain the more savage fighting among females where attributes such as weight (or size) did not carry any significance. In fact, high rank among females seemed to be attained by sheer tenacity and aggression regardless of any other factors. Rothe (1975) found no age or weight relationship with rank among males, but all the members in some of his groups of marmosets were related and may have fought out a rank order on different criteria.

Reproductive status of the female (i.e. whether or not she was cycling) might have some bearing on the disputes over rank order. In 5 out of 6 cases, the dominant female was among those which cycled 1-4 weeks before inclusion into the peer group and two of the fiercest disputes were between cyclic females. There was, however, one instance where the only previously cycling female did not become dominant. Nevertheless this does not necessarily weaken the arguement as the dominant female might have cycled more than 4 weeks prior to the formation of her peer group and this would not have been detected. This aspect requires further investigation.

5.5b The 'pair bond' and the monogamous breeding system of the marmoset

The pair-bonding behaviour between one male and one female and exclusivity of breeding typifies the monogamous system of the marmosets and the Callitrichidae in general, and contrasts with the multi-male groups or one-male harems of the generally polygamous Old World monkeys, such as macaques and baboons (Kleiman, 1977). However, as the latter author points out, "there are no more intensive socio-sexual interactions in species exhibiting long-term pair-bonding than in polygamous forms." The difference with the bonding in marmosets appears to lie with both the dominant animals suppressing or inhibiting any other pair-bonding behaviour between subordinate animals of their own sex and either their own dominant partner or subordinate animals of the opposite sex (Epple, 1967; Rothe, 1975; section 5.3h). In family groups of common marmosets incestuous sexual behaviour of any kind is extremely rare so long as both of the original parents are present (Rothe, 1975; section 5.3b). But, as soon as one of the parents dies or is removed, the offspring fight to fill both dominant positions and the remaining parent is 'ignored' (Rothe,in press). After the restructuring of the hierarchy, the new dominant brother and sister become the breeding pair (they were not co-twins) and the 'parents' of the group. The elusive nature of the restriction on sexual behaviour in family groups makes sexual suppression difficult to explain, but all the animals were related and relationships with and among the offspring had been formed from birth. Some form of incest taboo(s) might operate under these conditions (Rothe, 1975). In peer groups, however, or groups of unrelated adults (Epple, 1967; Rothe, 1975) the mechanism(s) are more precise. Rank order within each sex again determined which

male and female paired up and the relationship of the pair was maintained in at least two ways. Firstly, each dominant animal usually disrupted any sexual interactions between the dominant animal of the opposite sex and subordinates of its own sex. Secondly, subordinate animals might flee from the dominant animal of the opposite sex regardless of whether the approach was sexual or otherwise. Dominant animals (Male and Female 1) did not usually flee the sexual or aggressive approaches of the other.

However, initial sexual interactions in groups of unrelated animals could involve animals not destined to be dominant. This suggested that initial partner preference might be overruled by the strict dominance orientated mating system, and subordinate partners were either ousted or withdrew from most of the sexual interactions. Nevertheless, as Rothe (1975) found, the suppression of subordinate sexual behaviour in groups of unrelated marmosets was not as complete as in family groups, again suggesting that some form of incest taboo(s) was operating in these latter groups. But, as only the dominant female ovulates in peer groups, dominant males in groups of unrelated marmosets might only totally exclude subordinate males from mating with the dominant female around the time of ovulation. This was exactly what Rothe (1975) found.

The less restrictive nature of mating behaviour in groups of unrelated marmosets brought authors such as Epple (1967) and Rothe (1975) to question whether or not the monogamous social structure observed was just a product of captivity. However, field studies of the common marmoset (Stevenson, in press, a) and the closely-related tamarins (Dawson, 1976; Neyman, 1978) confirmed the monogamous system. Rothe (1975) has also suggested that in the common marmoset an 'emotional bond'

is a requirement for monogamy. But this criterion, however attractive in terms of human behaviour, is not useful because it does not have genetic consequences for the individuals, whereas mating exclusivity has.

5.5c An analogy between families and peer groups of marmosets

In a context of a monogamous species, the role of unrelated members of peer groups in raising the young of the dominant female is puzzling, especially as the former animals were able to breed within 1-4 months of separation from the dominant pair. Possibly, with captive marmosets, the artificial social structure of the peer group may mimic the monogamous family group, in which newborn young are a source of great interest, with the dominant pair becoming 'parents' and the other members of the group becoming their 'juveniles'. However, the analogy between family and peer groups ends when the young of the dominant female are weaned. In the peer groups, the dominant female attacked the other females and the subordinate animals were removed, leaving the dominant pair and their offspring. In the family groups of similar size, there was either, no violence following the birth of further offspring (n=12), or one of the older siblings attacked a younger one which had to be removed (n=4). As found by Rothe (1975), the parents were not involved in the expulsion of their offspring. The break-up of the peer groups might be due to the developing offspring disrupting the previous dominant-subordinate relationships. Marmoset offspring start to be incorporated into the group hierarchy following weaning (Rothe, in press.) Rothe (1975) also found that the increase in aggressive behaviour of the dominant female usually led to the break-up of his artificial groups of marmosets. In Rothe's groups these events also coincided with the increased sexual activity of the dominant female.

However, no such correlation was found in our peer groups (casual observations only) and they regularly broke up 1-3 months after the youngsters were born, if the young survived. By this time most of the dominant females were at least early pregnant and no fighting had occurred around the time of conception. In other colonies, the break-up of groups of unrelated marmosets was not as predictable as in ours (Epple, 1967; Rothe, 1975).

While the family groups are obviously more stable than groups of unrelated animals, youngsters are eventually expelled. As found by Rothe (1975), the parents were not directly involved. However, Poole, Stevenson and Sutcliffe (1978) observed mothers or fathers peripheralising daughters or sons respectively in 27% of cases, and Hampton, Hampton and Landwehr (1966) and Epple (1967) only found parents responsible for expulsions. Nevertheless, regardless of which mechanism predominates, older animals are usually responsible for banishing younger animals of the same sex from their natal group. This presumably forces these animals to join another group(s) or to form new groups.

5.5d Reproductive inhibition and the maintenance of the marmoset's monogamous breeding system

In groups of captive common marmosets only the dominant female became pregnant, similar to the findings of Epple (1967) and Rothe (1975). However, there appear to be two modes of reproductive inhibition. In family groups, while daughters can ovulate (section 5.3b) and sons can ejaculate spermatozoa (section 5.3 ; Rothe, (1975)), mating is almost exclusively limited to the parents. Some form of incest barrier(s) would seem to exist. This possibility was supported by one peculiar case from our colony when a breeding adult female was given an unrelated captive-bred male following the death of her previous mate. This male

proceeded to mate with her and her remaining daughter (550 days old). Both became pregnant, but so far only the daughter has produced a term pregnancy. The mother miscarried her first pregnancy but became pregnant again and helped to carry and care for her 'grandchildren'. As Rothe (in press) found, any barriers preventing offspring from breeding in family groups were destroyed when one of the original parents died or was removed.

In contrast, subordinate females in peer groups experienced complete suppression of fertility because they did not appear to ovulate (as determined by their low plasma progesterone concentrations). Measurement of oestrogen in urine samples collected from females in peer groups and female-female pairs also indicated ovarian cyclicity only in the dominant females (Lunn, in press). In fact, urinary oestrogen levels in subordinate females were typical of those found in ovariectomized females, suggesting that the ovaries of these animals were 'switched off'. In these cases, restrictions on subordinate mating behaviour are rather superfluous, as subordinate females cannot become pregnant. Finally, in contrast to Rothe's (1975) findings, the inhibition of subordinate female fertility did not extend outwith each caged group. Formerly subordinate females were able to breed successfully without being moved out of the room housing the dominant female.

On the other hand, there was little evidence to suggest whether there was suppression of fertility in subordinate males, as the dominant males did not completely exclude others from mating with the dominant female, and subordinate males were still capable of ejaculating spermatozoa. However, Rothe (1975) has found evidence of subordinate males failing to intromit and ejaculate in their groups. Subordinate

male fertility could be clarified if the paternity of the dominant female's offspring was determined. This has been accomplished in a troop of rhesus monkeys by blood typing the parents and offspring (Duvall, Bernstein and Gordon, 1976). In marmosets, individual differences cannot be found on the basis of human blood group substances such as A and B (Gengozian, 1964; Wiener, Moor-Jankowski and Gordon, 1967). Specific antisera reagents are required from reciprocal immunisations of red blood cells within and between species (Gengozian, 1972a, 1972b; Gengozian and Patton, 1972). Unfortunately such reagents were not available in Edinburgh. Nevertheless, as mentioned earlier dominant males might only totally exclude subordinates from mating with the dominant female when ovulation was imminent, such as in the immediate post-partum period (Hearn and Lunn, 1975), and Rothe (1975) has found such evidence. The dominant male did not become more aggressive towards subordinate males at these times, but he endeavoured to keep all of them away from his partner. It is noteworthy that the dominant female was not at all receptive to subordinate males on these occasions, re-emphasising the importance of the pair bond.

Therefore, regardless of whether the mechanism(s) underlying reproductive inhibition is behavioural and/or physiological, mature captive marmosets in family or unrelated groups only breed successfully if they are one of the parental or the dominant pair. The monogamous status-quo is thus maintained. Moreover, field studies have confirmed the stable parental pair and their recent offspring as the breeding core of marmoset and tamarin species (Dawson, 1976; Neyman, 1978; Stevenson, in press). Consequently any suppression of fertility in mature offspring remaining with their family will help prevent inbreeding and enhance any movement away from the family group into other groups or sub-groups, where suppression of fertility may be removed.

5.5e Female reproductive inhibition in marmosets in comparison with other species

In groups of unrelated marmosets subordinate females cease to ovulate, but what causes this suppression? It does not appear to be pheromonal or auditory as previously subordinate females can breed in cages next door to the original dominant female. They also have access to the same exercise cage, but at different times, and can observe each other on such occasions. Only when subordinates are in close physical and visual contact with unrelated animals does the anovulatory syndrome appear. It is possible that subordinate females under the stress of the constant close proximity of an unrelated dominant female may have elevated prolactin levels (hyperprolactinaemia) producing high-prolactin-amenorrhea similar to that found in women (Besser et al, 1972; Tyson et al, 1972; Thorner et al, 1974; van Look et al, 1977b; Baird et al, in press). This explanation fitted perfectly for the remaining subordinate female (Female 3) in 1 of the 3 peer groups tested before and after they were set up (Figure 5.17), and could explain the higher range of prolactin levels displayed by subordinates in well-established groups (Table 5.12). But in the latter groups, only 3 out of 12 subordinates had significantly higher prolactin levels than their dominant female (Female 1). Moreover, in the other two groups studied before and after they were set up, the remaining subordinate female (Female 2 in both) did not have elevated prolactin levels. Nonetheless Female 3 in these groups had wasted away and died following a peak of extremely high prolactin (Figure 5.18).

Bowman et al (1978; Keverne, in press) working with captive Talapoin monkeys (a polygamous species) have found definite evidence linking elevated prolactin levels and infertility, but only in the most subordinate female. The dominant females in each group did not exhibit

high levels of prolactin, but did show an LH surge in response to an oestrogen challenge. All Bowman et al's (1978, females were ovariectomized and their potential fertility was assessed by their LH response to an oestrogen surge, since the LH surge seems to be the trigger for ovulation. The most subordinate female in each group had elevated prolactin levels and did not respond to an oestrogen challenge. If the prolactin levels in the two types of female were reversed, their capacity to exhibit an LH surge followed suit. Intermediate ranking females did not fit readily into either category and may explain the ambiguous results found in some of the marmoset groups (e.g. Figure 5.18). As Bowman et al (1978; Keverne, in press) point out, these hormonal effects may be a result of changes in hypothalamic dopamine (Kordon and Ramirez, 1975; a neurotransmitter known to antagonise prolactin secretion) rather than a direct action of prolactin. But, there is still good evidence that prolactin alone can increase the refactory nature of the gonads to gonadotrophin stimulus and can affect the sensitivity of the hypothalamus to sex hormone feedback (rats: Fang, Retetoff and Rosenfield, 1974; McNeilly, Sharpe, Davidson and Fraser, 1978; human: Thorner et al, 1974; McNatty, Sawers and McNeilly, 1974; review: Friesen, McNeilly and Lancrajan, in press). However neither possibility can explain the reproductive inhibition experienced by the second-highest ranking female marmosets. Perhaps their circadian secretion patterns of prolactin have been slightly altered. Circadian patterns of prolactin secretion are known to exist in several species (Nicoll, 1973), including Man (Robyn et al, 1977) and there is evidence that in seasonally breeding animals such as sparrows (Meier and Martin, 1971; Nicoll, 1973) and sheep (Lincoln, McNeilly and Cameron, 1978) that natural changes in the timing of these patterns

can induce reproductive inhibition. Lowest ranking female marmosets may just show this type of effect to a greater extent than females of intermediate rank.

Keverne

Bowman et al (1978;/in press) found a relationship between cortisol levels and female reproductive inhibition, unlike the results in marmosets (Table 5.13). Plasma cortisol was significantly higher in the subordinate female talapoin. Adrenal glands from subordinate female marmosets were also not significantly larger than those from actively breeding females (S. F. Lunn, personal communication). From their work on male squirrel monkeys, Saimiri scuireus, Manogue, Leshner and Candland (1975) found that dominant animals always showed the greatest adrenal reaction to stress while exhibiting lower pre-stress levels of cortisol than subordinates. While no equivalent information is available on females, perhaps this type of reaction confuses any possible relationship of adrenal activity with marmoset reproductive inhibition, because their erratic prolactin results possibly indicate that animals are stressed or excited in the colony as a whole by unrelated event(s) preceding blood sampling.

In other primates (notably polygamous species), the effects of dominance on female fertility are partly inhibitory, as in Gelada baboons, Theropithecus gelada (Dunbar and Dunbar, 1977) and rhesus monkeys M. mulatta (Drickamer, 1974) in the wild, and in captive olive baboons, P. anubis (Rowell, 1970). These results contrast with those of Keverne (in press), and perhaps in the intact lowest-ranking female primate the inhibition is not as complete as in the ovariectomized (oestradiol-implanted) female of similar status. However, in tree shrews, T. belangeri, subordinate females suffer complete suppression of fertility only with increased density of population (von Holst, 1969).

Such a mechanism, also found in rodents (for a review see Brain, 1971; Brown, 1975, p. 119) does not seem to operate in marmosets. In other monogamous species such as the dwarf mongoose, Helogale parvula (Rasa, 1973), the wolf, Canis lupis (Mech, 1970; Woolpy, 1970; Zimen, 1976) and the African Cape hunting dog, Lycaon pictus (Frame and Frame, 1976) what scanty physiological evidence exists suggests that subordinate females, while not breeding, still cycle. They are only prevented from breeding by the active suppression of their sexual behaviour by the dominant animal(s). However, in the example from the dwarf mongoose (Rasa, 1973) the oestrous cycles of subordinate females may have been anovulatory and the conditions under which they became pregnant did not exclude them from ovulating through a reflex response to mating. The menstrual cycles of subordinate female wolves (Zimen, 1976) might be similarly anovulatory. At least in the human, vaginal bleeding does not necessarily indicate an ovulatory cycle (van Look et al, 1975; Baird et al, in press).

The mechanism(s) by which social dominance in animal societies, especially primate societies, inhibits reproduction in the female requires investigation and the captive female marmoset may serve as a good model. If, as indicated by Bowman et al (1978; Keverne, in press), female reproduction inhibition in primates has a physiological basis, the latter aspect may have the potential as a future method of contraception.

5.6 Summary

In any captive marmoset group, only the dominant female reproduced.

5.6a Family groups

There was no clear rank order because dominant-subordinate interactions were rare. While apparently only the mother and father bred,

their maturing daughters did ovulate and their sons could ejaculate spermatozoa. However, sexual behaviour was almost exclusively limited to the parents.

5.6b Peer groups

(1) In newly-formed groups of unrelated animals, clear rank orders were usually achieved because of frequent dominant-subordinate interactions. The dominant male and female ranked as dominant to all the others, formed a pair bond and attempted to prevent any other sexual relationship.

(2) The dominants' pair bond was significantly associated with their huddling together, following each other and remaining within arm's length of one another.

(3) After the first 2-3 days, usually after the removal of one of the subordinates because of persistent attacks from one or more dominant animals, the frequency of dominant-subordinate interactions dropped dramatically.

(4) With well-established peer groups, like the families, there was no clear rank order because dominant-subordinate interactions were rare. However, the dominant pair were identified by their association (male following the female, etc.).

(5) Among peer group males, older and heavier animals significantly tended to hold high rank whereas no such correlation was found with females. Nevertheless previously cyclic females did tend to become dominant.

(6) Subordinate females experienced complete suppression of fertility because they ceased to ovulate. There was little evidence to suggest whether the fertility of subordinate males was suppressed because they

could still ejaculate spermatozoa and dominant males did not completely exclude others from mating with the dominant female.

(7) Because of fighting, peer groups had to be broken up after the young of the dominant female were weaned, unlike families.

5.6c General points

(1) It was therefore concluded that reproductive inhibition in peer groups was mediated by physiological and behavioural means whereas only behavioural restrictions operated in families.

(2) As free-living groups of marmosets have a similar social structure to captive groups, it was suggested that any suppression of fertility in mature offspring would help prevent inbreeding and enhance any movement away from the family group into other groups or sub-groups, where suppression of fertility may be removed.

(3) There was some evidence of elevated prolactin levels accompanying the anovulatory condition in subordinate peer group females, resembling the infertile hyperprolactinaemia syndrome in women. The captive female marmoset may therefore serve as a good model to study human infertility and to investigate the mechanism(s) by which social dominance inhibits female reproduction in primate societies.

CHAPTER 6: THE EFFECT OF NEONATAL EXPOSURE TO TESTOSTERONE ON THE DEVELOPMENT OF FEMALE MARMOSETS AND THE COMPARATIVE EFFECTS OF ORCHIDECTOMY AND OVARIECTOMY

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6.1 Introduction

Male marmoset monkeys (Abbott and Hearn, 1978), rhesus and pig-tailed macaques (Robinson and Bridson, 1978) and humans (Forest et al, 1973a,b) all show high circulating levels of testosterone in early infancy, but females do not. The function of this neonatal testosterone is as yet unknown, but may be involved in the differentiation and/or maturation of hypothalamus-pituitary-testis hormonal feedback and brain centres controlling behaviour. Genetic female marmosets implanted with testosterone during early infancy may provide a means to test the physical, hormonal and behavioural consequences of neonatal exposure.

The common marmoset monkey (described in Chapter 1) provides a unique model for studying sexual differentiation and sexual development in a primate. The marmoset produces dizygotic twins 50-70% of the time (Hampton, Gross and Hampton, 1978; Lunn and Hearn, 1978; Ogden et al, 1978). The shared placental circulation seen in this species does not lead to the freemartin condition in females born co-twin to a male (Wislocki, 1939) even though such twins are XX/XY haematopoietic chimeras (Benirschke and Brownhill, 1962; Benirschke et al, 1962; Gengozian et al, 1964; Benirschke and Layton, 1969). The testes and ovaries of males and females are seemingly not chimeric and have XY and XX genotypes, respectively (Ford and Evans, 1977). The evidence for XX spermatagonia and XX spermatocytes in the testes of male marmosets (Benirschke and Brownhill, 1963; Egozcue, Perkins and Hagemenas, 1968) and XY oocytes in females (S.M.H. Hampton (1970) cited by Ford and Evans, 1977) is far from definitive and could equally be explained as a mis-interpretation of the chromosomal preparation or as a mis-interpretation of the cells as spermatagonia, etc.

(Ford and Evans, 1977). The female marmoset fetus therefore is quite unaffected by early placental vascular connections to a male co-twin, unlike the fate of similarly co-joined females in cattle, sheep, goats and possibly pigs (Marcum, 1974). Nonetheless, it is not known how she achieves this 'immunity'. It is therefore possible that the marmoset has delayed sexual differentiation until after birth. However, the gonads and internal and external genitalia of male and female marmosets are well formed at birth,implying some difference in sexual development before birth. Yet, this difference may be localized to the immediate area surrounding the gonads and may not involve the general circulation (see section 6.4 and Chapter 7 for further discussion).

Hence, in order to discover to what extent neonatal androgen treatment could influence sexual differentiation, newborn female marmosets were implanted with testosterone and the effects on their external genitalia and their playful and sexual behaviour were observed. Newborn males and females were also gonadectomized for comparison. The pattern of their circulating LH concentrations was followed to determine whether the hypothalamic-pituitary unit was capable of responding to the removal of negative feedback (i.e. steroid secretion from the gonads) at an early age. If the males responded to castration with an LH rise in the neonatal period, this would mean that neonatal testosterone was biologically active, at least at the level of the hypothalamus and pituitary. In the human male much of the neonatal testosterone is considered biologically inactive because it is bound to a sex hormone binding protein in the blood (Forest et al, 1973a, 1974a).

6.2 Procedure

6.2a General methods

Details of the animals, surgery, vaginal washings, collection of blood samples and radioimmunoassays have been given in Chapter 2.

6.2b Testosterone and gonadectomy treatment

Five female marmosets were each implanted subcutaneously one to four days after birth with 25mg. of crystalline testosterone (see Chapter 2). Their co-twins or triplets were sham-operated in a similar manner (Table 6.1), but no implant was inserted. Pairs of animals in male-female (n=8), male-male (n=3) and female-female (n=3) twin sets were also used as non-experimental controls.

When the implants were removed they were dried in an oven at 70°C. for 24hr. Their weights were then compared with those obtained before implantation. The total dosage of testosterone received by each was calculated (Table 6.1) and, assuming a constant release of testosterone, the daily dosage was expressed in terms of mg/kg. body weight.

Three male and female marmosets were each bilaterally gonadectomized one to four days after birth (see Chapter 2). Their co-twins were sham-operated in a similar manner (Table 6.2). The same non-experimental controls for the testosterone implant study were used (see above). One female was also bilaterally ovariectomized prepubertally at 180 days of age with no co-twin control (Table 6.2).

6.2c Blood sampling schedule

Females receiving testosterone implants (e.g.Plate 1), and their co-twin or triplet controls were bled at 10-20 day intervals while under 100 days of age, and at 50-100 day intervals thereafter (0.5ml. samples).

PLATE 1: A 50-day old female marmoset receiving testosterone

Between 400-600 days of age they were bled at 2-3 day intervals for two months, on at least two occasions, to see if they showed any ovulatory activity (plasma progesterone concentration of 20ng/ml.; Chapters 2 and 4).

Gonadectomized animals were bled at approximately 30-100 day intervals throughout (0.3-0.7ml. samples).

6.2d Families: Behaviour recorded and the statistical analysis

The families were observed as described in Chapter 2. Only bouts of social play between experimental or control sets of twins were analysed. Social play (see Chapter 2) was recorded under the following categories:

rough-and-tumble

chase

hide-and-seek.

Each play category was quantitatively assessed after each observation (a surviving triplet in one set was not included). For example, the total rough-and-tumble play initiated by a control female was subtracted from the total rough-and-tumble initiated by her androgenized co-twin sister. The resulting positive or negative differences ($\pm\Delta R$) from every observation were then analysed using the Sign Test (one-tailed; Siegel, 1956) and zero values were ignored. As a positive value ($+\Delta R$) was therefore given to all the androgenized females, a similar sign was given to the scores of males in normal male-female twin pairs and all experimental animals. In the twin pairs of the same sex, a positive value was given to the twin which accumulated the largest number of differences by initiating more rough-and-tumble play than its co-twin in a larger number of observations. The negative value ($-\Delta R$) was assigned to the other twin. A similar analysis was carried out for chase (ΔC) and hide-and-seek play (ΔH), and each animal kept its + or - sign from the rough-and-tumble analysis.

6.2e Observation of peer groups and behaviour recorded

Experimental and control animals were transferred from their families to peer groups, as described in Chapters 2 and 5. The groups were observed for the first three days after they were set up and the behaviour recorded was identical to that previously described. Rank (i.e. dominant or subordinate status) and pair-bonding were also determined on previous criteria (Chapter 5).

After the subordinate androgenized females had been used in the behavioural trials, they were removed from their original peer group and established in others. This was done in an attempt to elevate their previous rank to the dominant female position and thus allow them to breed.

6.2f Behavioural trials of unfamiliar animals, the behaviour recorded and the statistical analysis involved

After the experimental and control animals were placed in peer groups, sets of normal (n=5), androgenized (n=5), and ovariectomized (n=3) females and sets of normal (n=5) and orchidectomized (n=3) males were observed in randomised combinations of unfamiliar pairs of animals. Each pair was observed in the observation room for 15 min. between 09:30 and 16:30h. and neither animal was observed in any further trials for at least two hours. The sets of normal males, and normal and androgenized females were balanced with respect to their dominance rank in their peer groups (3 dominant and 2 subordinate in each set; in the first series there were only 2 dominant females in the androgenized group). After the initial two series of trials involving normal males and females and androgenized females (see below), the group of 'normal' females was split into two and the numbers in each again made up to five. One group contained females born with a co-twin sister and the other contained females born with a co-twin male. These two groups were then re-tested in a third series of trials along with the normal males (but not the androgenized females) to see if

the sex of the females' co-twin had had any effect on their adult behaviour. Only the group of females with co-twin sisters was used for comparison with gonadectomized animals.

The initial two series of trials (i.e. each pair combination was observed twice) were carried out on normal males and females and androgenized females with an interval of one month. In the next series, when the group of normal females was split up, and when gonadectomized animals were tested with normal males and females, only one observation of each possible combination of pairs was carried out.

The following behavioural categories were recorded (see Chapter 2 for details):

Sexual behaviour: pelvic thrust
mount
attempt mount
pursuit courtship
passive courtship
head turn
limbs and body motionless
crouch
accept mount
reject mount

Aggression and submission:

'threaten and pursue' : combining vocal threat with follow attack
'submission' : combining all submissive categories (Chapter 2).

The numbers of control and experimental animals displaying each behaviour were compared by using Fisher's Exact Test (Siegel, 1956).

6.3 Results

6.3a Testosterone dosage and the effects of experimental treatment on body weight

The implanted females were exposed to testosterone doses of between 3 and 10mg/kg. body weight per day. The large variation in dose was due to the rapid growth of these animals but, like the animals under other experimental regimes, they did not exceed the weight of their controls (Tables 6.1 and 6.2).

Circulating concentrations of testosterone were maintained at about 100 ng/ml in implanted females and these concentrations fell below the sensitivity limit of the assay when the implants were removed (Figure 6.1). Plasma testosterone concentrations in control females remained immeasurable throughout.

6.3b The effects of neonatal exposure to testosterone on female genitalia

By 40-50 days after implantation the clitoris of the androgenized females had become significantly hypertrophied and masculine scrotal and pudendal pad swelling was very marked (Figure 6.2). The vaginal opening was obscured by the folds of the glandular pudendal pad but was not obstructed. In comparison, in a normal male of this age the penis was still concealed inside the prepuce. The scrotum was well defined but poorly developed and the testes were usually undescended. In a normal female the external genitalia were not grossly different from those of the normal male, and the clitoris and vaginal opening were tiny and obscured by the labia.

At 350 days of age, 300 days after the implants were removed, the androgenized females still possessed genitalia that were intermediate

TABLE 6.1: Age, body weight and the total dose of testosterone received in implanted female marmosets with their respective co-twin or triplet controls.

(a) Before and after implantation.

FAMILY	AGE (DAYS)	IMPLANTED FEMALES		SHAM-OPERATED CONTROLS	
		BODY WEIGHT(GM.)	TOTAL DOSE OF TESTOSTERONE (mg)	ANIMALS	BODY WEIGHT (GM.)
1	4-53*	30-90	-*	FEMALE^{S}	29-68
2	1-51	30-60^{8}	14.8	FEMALE^{S}, FEMALE^{+S}	28-69, 30-68
3	4-55**	30-97	14.9	FEMALE^{+}	31-$^{+}$
4	2-52	30-96	14.3	FEMALE‡	32-100
5	4-54	32-91	16.5	FEMALE^{+}, MALE$^{\ddagger S}$	29-$^{+}$, 27-80

(b) Over 550 days of age

FAMILY	BODY WEIGHT (GM.)	
	ANDROGENIZED FEMALES	SHAM-OPERATED CONTROLS
1	331	342
2	326	308
3	241	-$^{+}$
4	352	306
5	278	296(M)

* Implanted for 49 days; implant lost *in situ*

** Implanted for 51 days

S Sham-operated only at implant removal

8 Only triplets to survive, hence reduced body weight

\+ Died pre-pubertally

$\ddagger$ Died post-pubertally

(M) Male

TABLE 6.2: Age and body weight of gonadectomized male and female marmosets with their respective co-twin or triplet controls.

(a) Orchidectomized males.

FAMILY	AGE (DAYS)	BODY WEIGHT (GM.)	
		ORCHIDECTOMIZED MALES	SHAM-OPERATED CONTROLS
1	4- 550	30-330	30-316
2	3- 550	33-320	30-365
3	3- 550	30-318	29-+

(b) Ovariectomized females

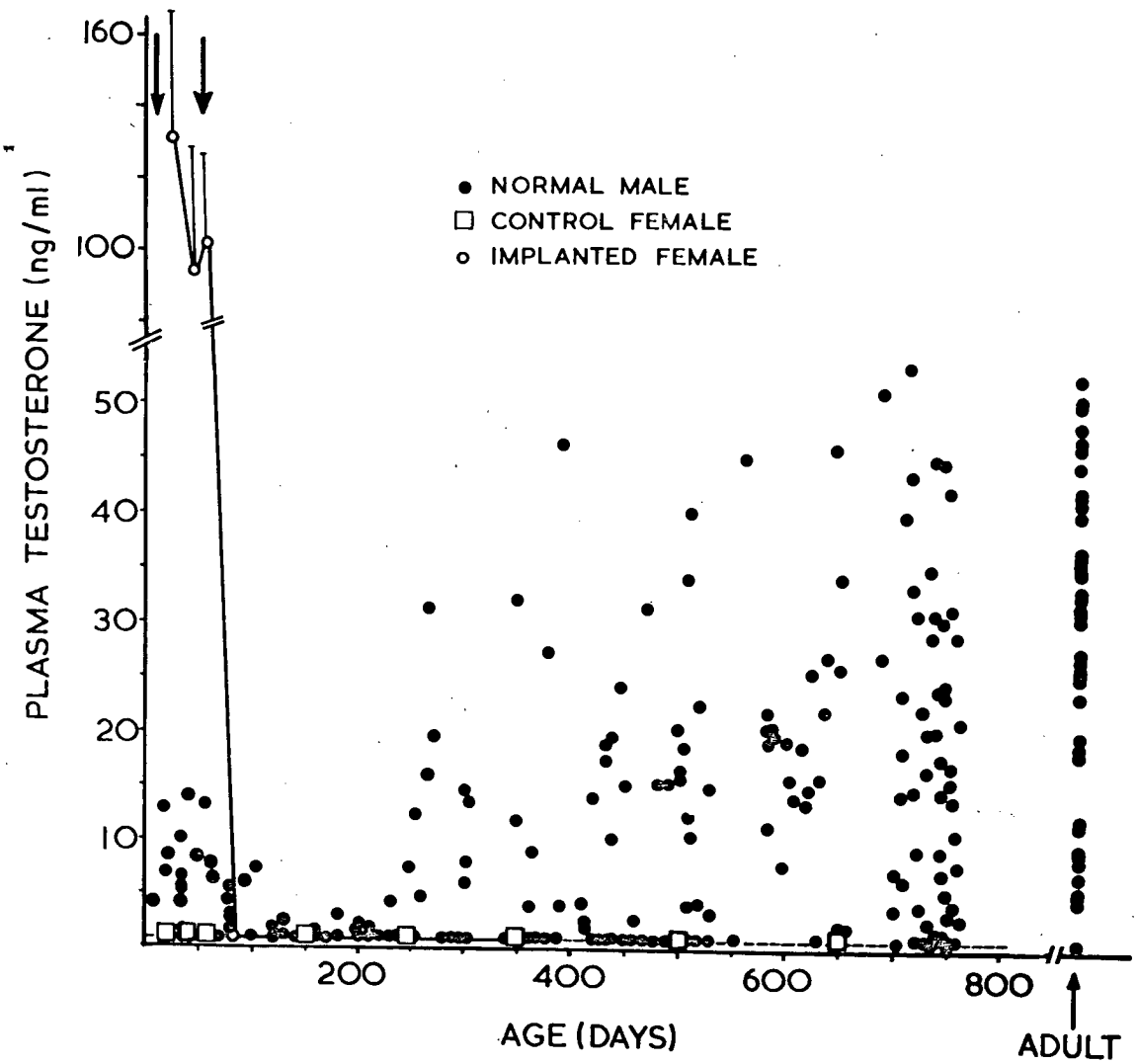
FAMILY	AGE (DAYS)	BODY WEIGHT (GM.)	
		OVARIECTOMIZED FEMALES	SHAM-OPERATED CONTROLS
1	3- 550	29-274	28-274
2	3- 550	NA-268	NA-317(M)
3	4- 550	30-284	32-257
4	550	276	-

\+ Died pre-pubertally

NA Data not available

(M) Male

FIGURE 6.1: Circulating concentrations of testosterone in implanted females (Mean ± S.D.) and their controls superimposed on top of the concentrations found in normal developing males.



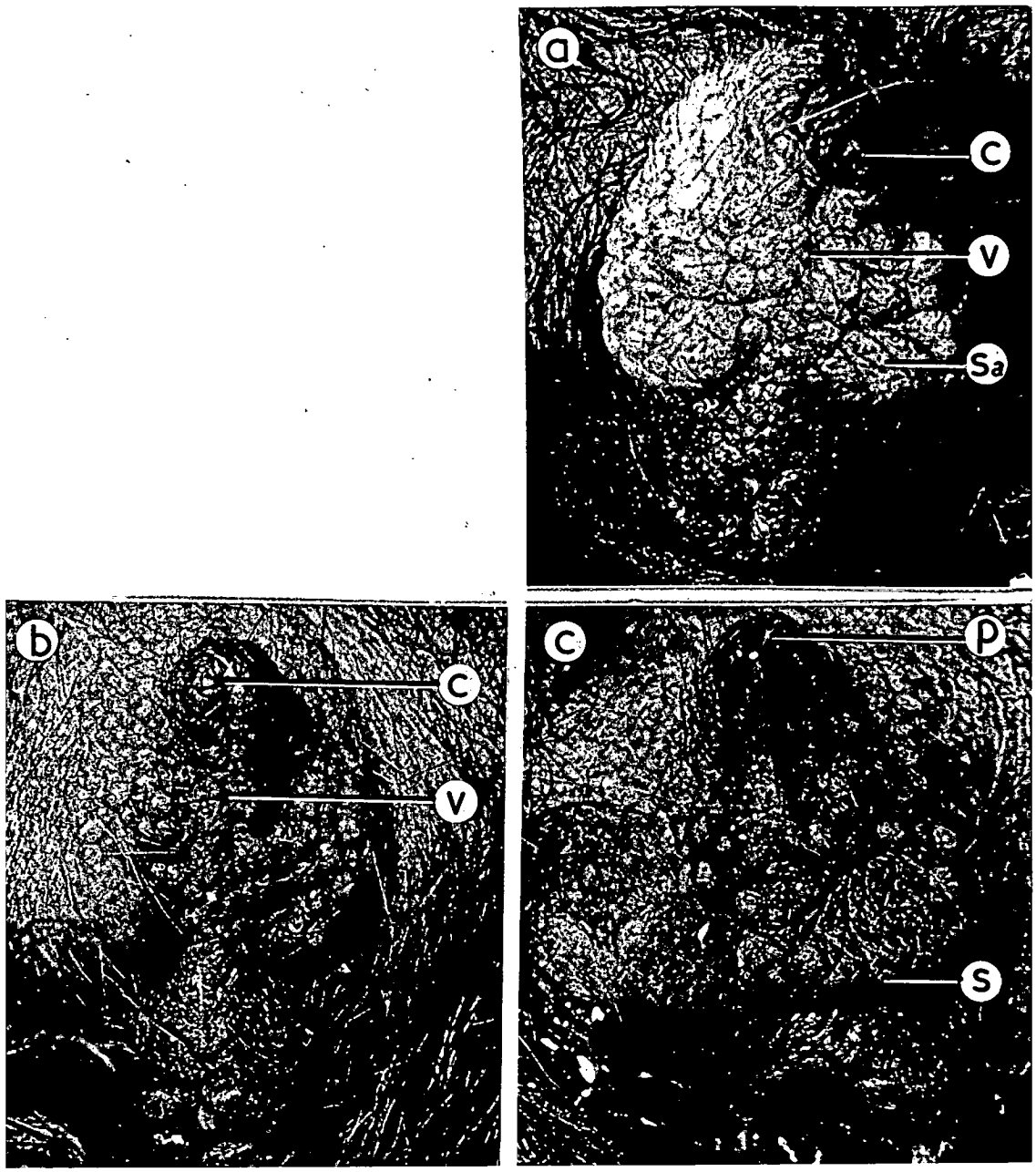


FIGURE 6.2: The external genitalia of (a) an androgenized female marmoset, compared to those of (b) the normal female, and (c) male, at 50 days of age. The perineal area is shown and the light-coloured, nodular pudendal pad of the androgenized female highlights her genitalia. In (a), the markedly hypertrophied clitoris (c) protrudes well beyond the labia, the vaginal opening (v) is present, but obscured, and the scrotal area (Sa) is swollen, but empty. In (b), the clitoris (c) and the vaginal opening are obscured by the labia. In (c), the penis (p) is obscured by the prepuce and the scrotum (s) is empty. (a-c) are eight times their normal size.

between those of a normal male and a normal female (Figure 6.3). The androgenized females displayed a hypertrophied clitoris, and some swelling of the empty scrotal area and the pudendal pad. The control females showed none of these features and the maturing male now possessed a well-developed penis and scrotum into which the testes had descended. The genitalia of the androgenized females did not subsequently change their appearance.

6.3c Effects of ovariectomy and orchidectomy on genital development

The development of female external genitalia was little affected by bilateral ovariectomy (Figure 6.4). However, bilateral orchidectomy reduced the size of the pudendal pad in the male, and the penis and empty scrotum were relatively poorly developed (Figure 6.4). The development of the nodular surface of the pudendal pad was apparently normal in both sexes (Figure 6.4). These nodules are normally associated with sebaceous scent glands used to mark objects and cage mates (Sutcliffe and Poole, 1978).

6.3d The effects of the neonatal treatments on prepubertal play behaviour

In normal male-female twin pairs, males initiated more rough-and-tumble play than females when the twins played with each other (Table 6.3). In male-male and female-female twin pairs there was no difference in the amount of rough-and-tumble initiated between each twin. Nevertheless, androgenized females,with female co-twin controls, initiated much more of the rough-and-tumble play, similar to the situation in normal male-female twin pairs (Table 6.3). Yet, on the one occasion when the control twin was male, the androgenized female did not initiate significantly more rough-and-tumble play.

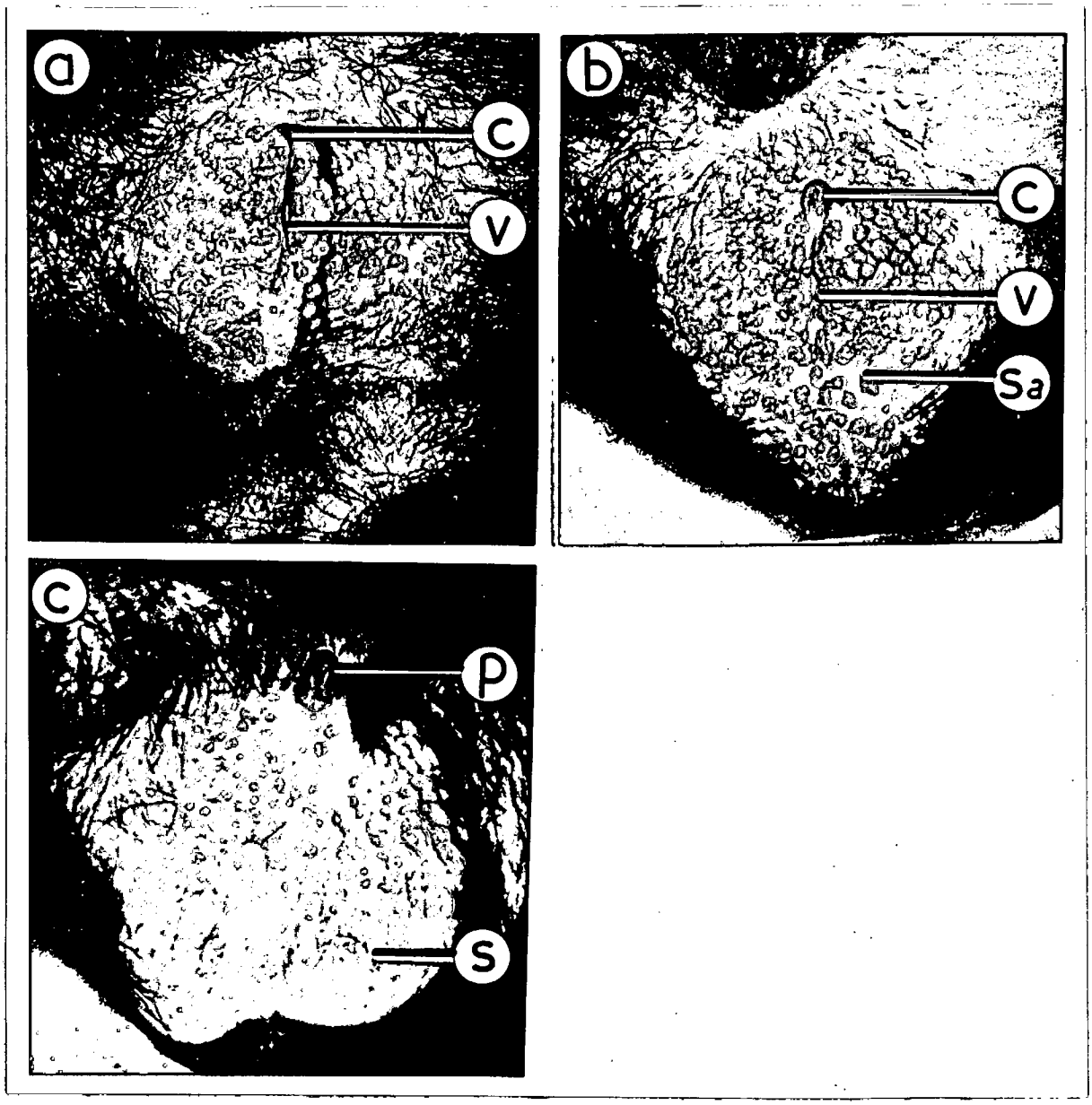


FIGURE 6.3: The external genitalia of (a) an androgenized female, 300 days after removal of the implant, compared to those of (b) the normal female, and (c) male, at 350 days of age. The perineal area is shown and the light-coloured, nodular pudendal pad highlights the genitalia. In (a), the clitoris (c) of the normal female is obscured by the labia and the vaginal opening is shown. In (b), the hypertrophied clitoris (c) of the androgenized female still protrudes between the labia, the vaginal opening (v) is present, and there is still some slight swelling of the empty scrotal area (Sa) and the pudendal pad. In (c), the penis (p) of the maturing male protrudes through the prepuce and the well-developed scrotum (s) contains testes. (a-c) are twice their normal size.

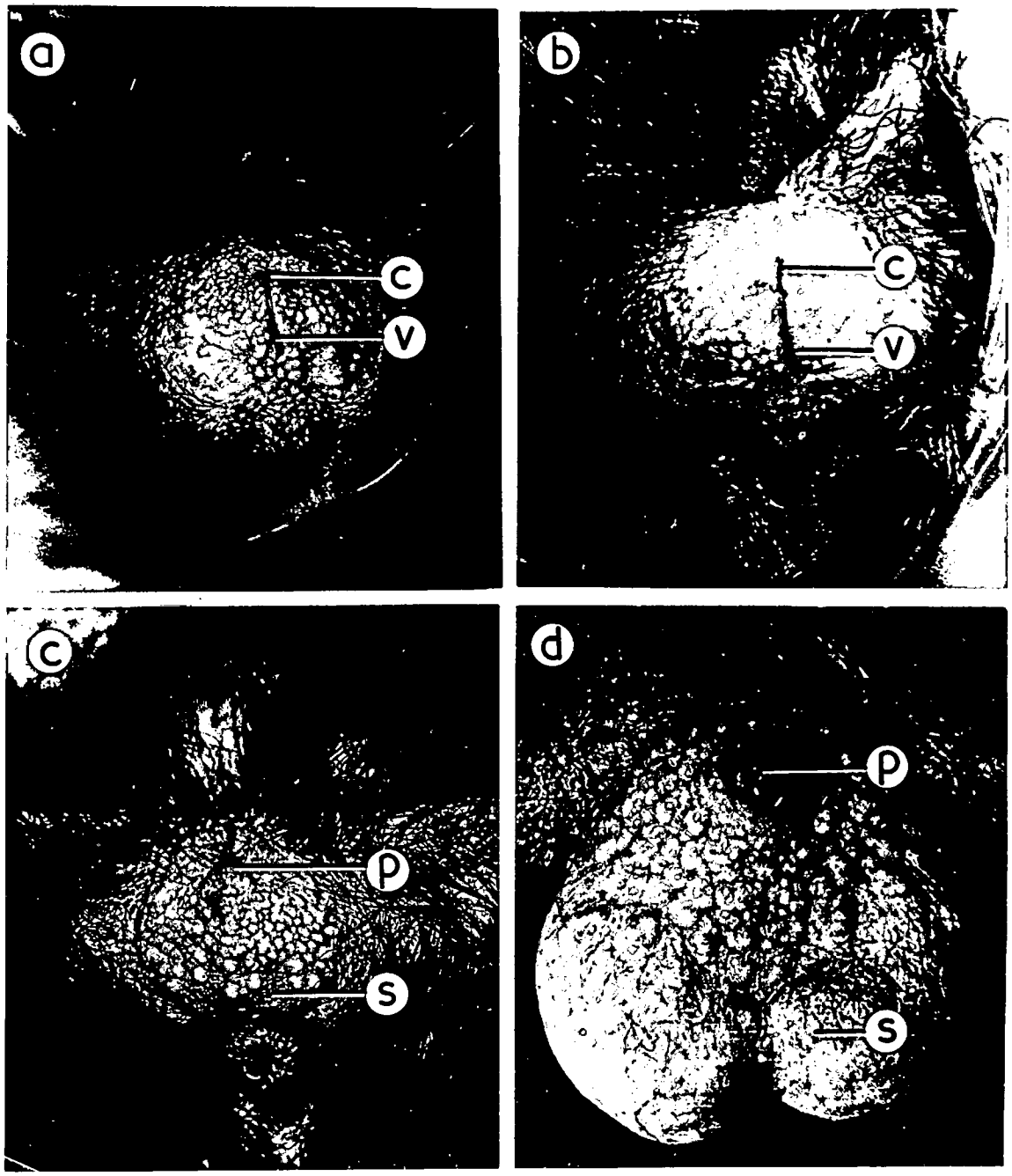


FIGURE 6.4: The external genitalia of (a) an ovariectomized female, (b) a normal female, (c) an orchidectomized male, and (d) a normal male when adult. The perineal area is shown and the light-coloured pudendal pad highlights the genitalia. In (a) and (b), the clitoris (c) is fairly well obscured by the labia and the vaginal opening is shown. In (c), the poorly-developed penis (p) is barely visible through the prepuce, and the scrotum (s) is empty. In (d), the penis (p) of the normal male is visible through the prepuce and the scrotum (s) contains testes. (a-c) are twice, and (d) is three times the normal size.

TABLE 6.3: Analysis of rough-and-tumble play between marmoset co-twins in their family groups.

TREATMENT	ANIMAL	ΔR SIGN	NO. OF TWIN SETS	ΔR +	ΔR −	TOTAL ΔR
Testosterone implant	Female A Female C	+ −	4	27***	1	28+
Testosterone implant	Female A Female C	+ −	1	4	2	6
None	Male N Female N	+ −	8	22*	9	31
None	Male N Male N	+ −	3	12	7	19
None	Female N Female N	+ −	3	11	6	17
Orchidectomy	Male E Male C	+ −	3	8	8	16
Ovariectomy	Female E Female C	+ −	2	5	3	8
Ovariectomy	Female E Male C	+ −	1	0	3	3

+ Triplet not included

A = androgenized

C = sham-operated control

N = non-experimental

E = experimental

* $p < 0.02$

*** $p < 0.001$

The frequency of rough-and-tumble play displayed between twins varied widely from family to family (e.g. the 5 families containing androgenized females, Figure 6.5). There was no consistent tendency to increase or decrease the frequency of this type of play as the animals aged. Notably in family 3, where a triplet survived until 100 days of age, the androgenized female initiated more of the rough-and-tumble with her as well as the sibling that lived longer (Figure 6.5).

Normal males with orchidectomized male co-twins did not initiate more rough-and-tumble when the twins played together (Table 6.3). Normal females with ovariectomized female co-twins and a normal male with an ovariectomized female co-twin behaved similarly.

There were no significant differences in the initiation of chase or hide-and-seek in any of the twin pairs (Tables 6.4 and 6.5).

6.3e The effects of the neonatal treatments on behaviour in peer groups

When housed in peer groups, three of the five androgenized females initially displayed (on Days 1 and 2) components of male sexual behaviour (see below). All three displayed pursuit courtship towards normal males and females, one violently attacked and pursued any male attempting to mount her, and one also attempted to mount normal males and females. Subsequently, two of these three animals became the dominant females in their groups and formed a 'pair-bond' with the dominant male in their group. The dominant male and female in a group normally form a pair bond and exhibit intimate and unhurried courtship and copulatory behaviour. Spermatozoa have been removed from the vaginal flushings of the two dominant androgenized females. The remaining three androgenized females ranked as subordinate to the dominant female in their group and displayed no further sexual behaviour.

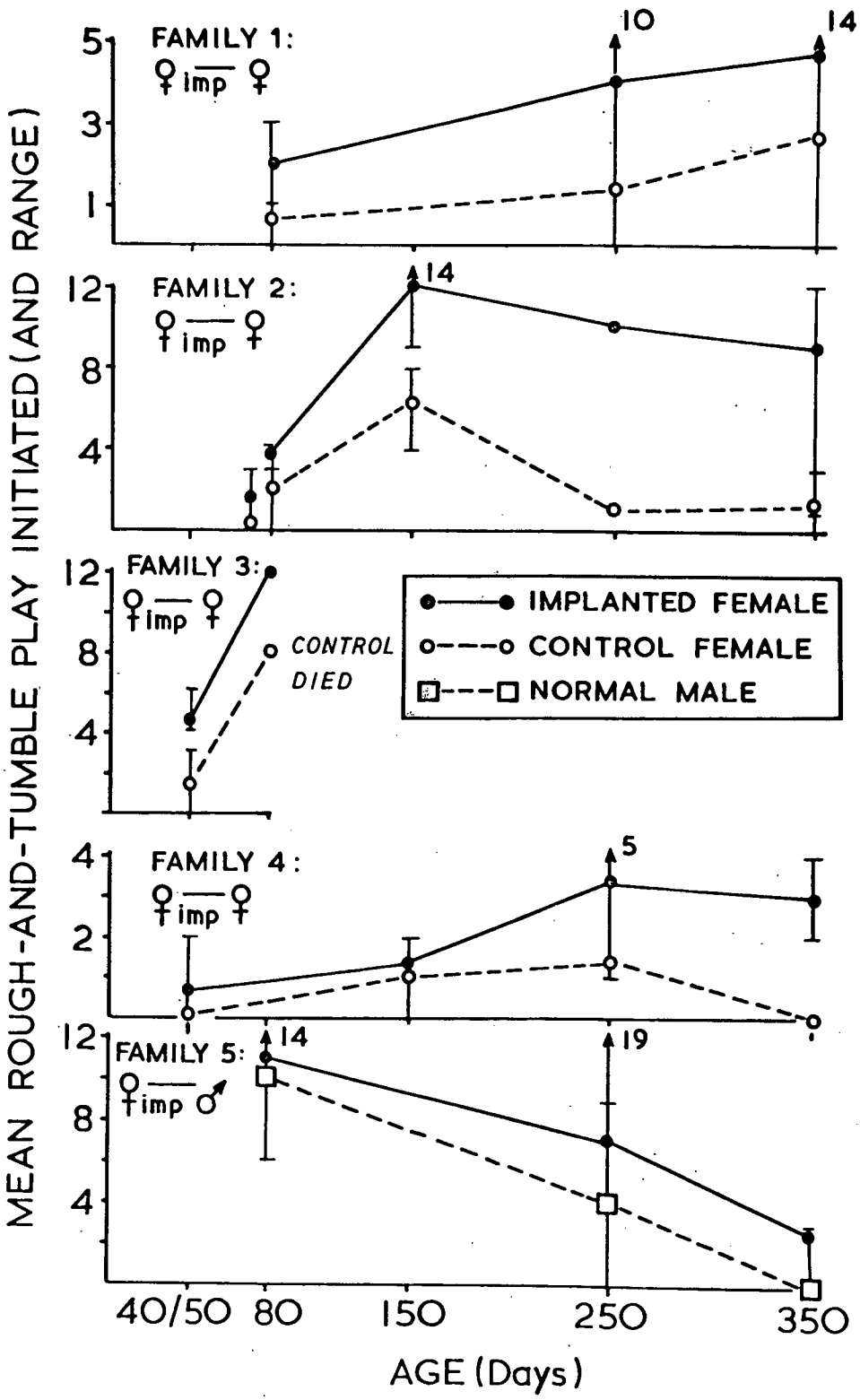


FIGURE 6.5: The mean frequency of rough-and-tumble play between androgenized females and their co-twin or triplet controls in their family groups.

TABLE 6.4: Analysis of chase play between marmoset co-twins in their family groups.

TREATMENT	ANIMAL	Δ C SIGN	NO. OF TWIN SETS	Δ C +	Δ C −	TOTAL Δ C
Testosterone implant	Female A Female C	+ −	4	7	5	12+
Testosterone implant	Female A Female C	+ −	1	2	1	3
None	Male N Female N	+ −	8	14	8	22
None	Male N Male N	+ −	3	3	1	4
None	Female N Female N	+ −	3	2	8	10
Orchidectomy	Male E Male C	+ −	3	8	4	12
Ovariectomy	Female E Female C	+ −	2	5	1	6
Ovariectomy	Female E Male C	+ −	1	2	0	2

+ Triplet not included

A = androgenized

C = sham-operated control

N = non-experimental

E = experimental

TABLE 6.5: Analysis of hide-and-seek play between marmoset co-twins in their family groups.

TREATMENT	ANIMAL	Δ H SIGN	NO. OF TWIN SETS	Δ H +	Δ H -	TOTAL Δ H
Testosterone implant	Female A Female C	+ -	4	5	7	12+
Testosterone implant	Female A Female C	+ -	1	2	0	2
None	Male N Female N	+ -	8	8	9	17
None	Male N Male N	+ -	3	2	2	4
None	Female N Female N	+ -	3	7	7	14
Orchidectomy	Male E Male C	+ -	3	5	3	8
Ovariectomy	Female E Female C	+ -	2	2	0	2
Ovariectomy	Female E Male C	+ -	1	1	3	4

+ Triplet not included

A = androgenized

C = sham-operated control

N = non-experimental

E = experimental

No spermatozoa were recovered from vaginal flushings of these females. Both surviving control female co-twins became dominant in their groups. One of the subordinate androgenized females became dominant in a group after a later reshuffle of animals. None of the three castrated males became dominant, whereas both surviving controls did. Two of the castrated males had been in two and three peer groups, respectively. Such males also never showed any sexual behaviour. Two out of four of the ovariectomized females became dominant as well as one of the two female controls. One of the subordinate ovariectomized females had held her status in two different groups. The dominant ovariectomized females copulated normally and spermatozoa were found in their vaginal flushings. No sexual behaviour was observed with the subordinate females.

Fighting was more severe in peer groups containing experimental animals, but the number of dominant-subordinate interactions was relatively similar to that in normal groups. Squabbles would erupt for up to two weeks after the groups were established. Such prolonged aggression was not observed earlier in groups of normal animals (Chapter 5).

6.3f The effects of the neonatal treatments on sexual behaviour

6.3f(i) Normal males, normal females and androgenized females

The results from both series of behavioural trials are summarised in Figure 6.6. Normal males displayed masculine copulatory and courtship behaviour and did not display any female sexual behaviour. Normal females showed feminine copulatory and courtship behaviour and only one displayed the pursuit courtship usually observed in males. Androgenized females, on the other hand, displayed components from both male and female sexual behaviour. Yet, when compared with normal males,

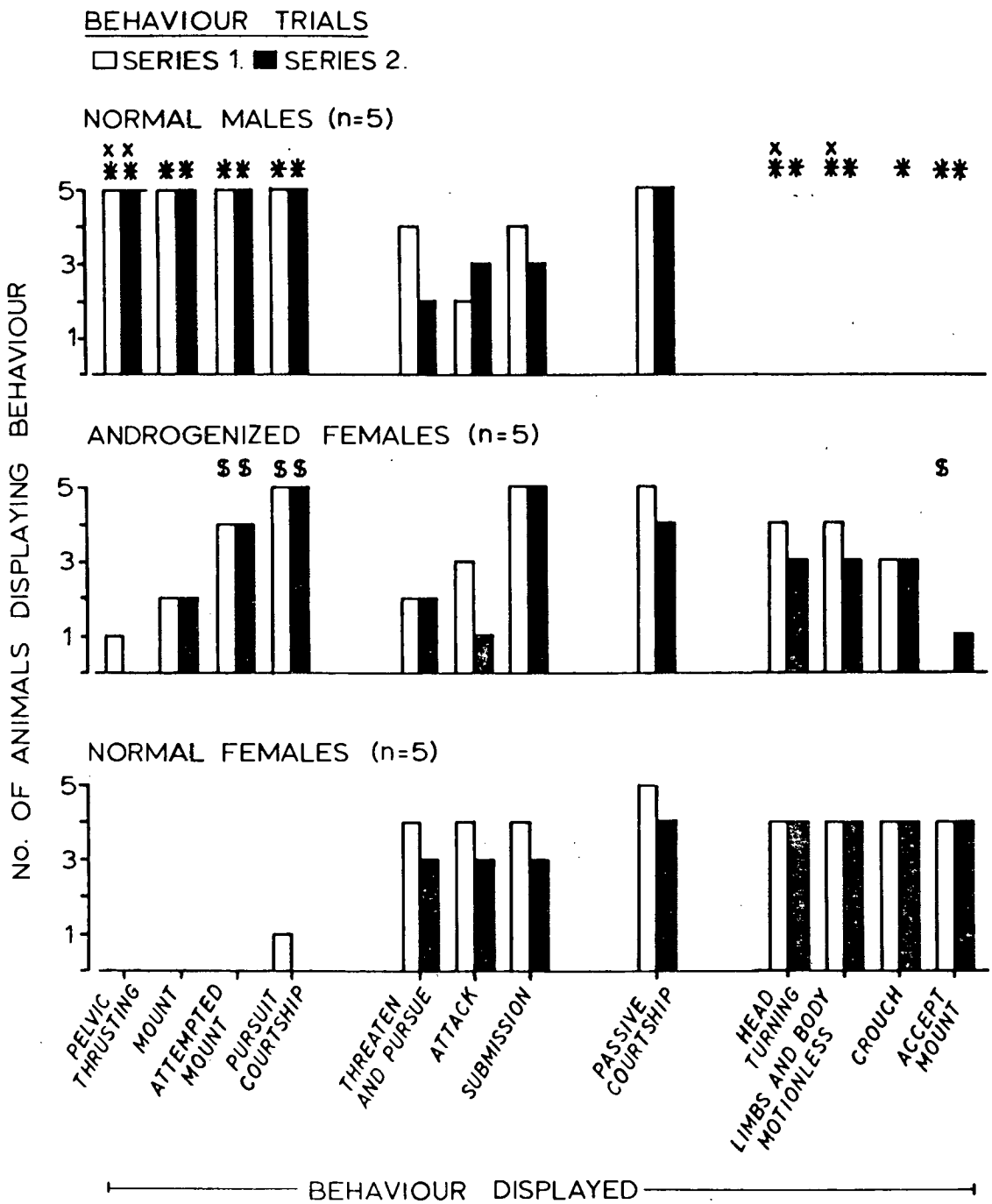


FIGURE 6.6: Masculine and feminine behaviour displayed by normal male, androgenized female and normal female marmosets during 15-min. behavioural trials. Significant differences ($p < 0.05$) were found between : (*) normal males and normal females, (X) normal males and androgenized females, and ($) normal females and androgenized females.

significantly fewer exhibited pelvic thrusting behaviour and, unlike normal females, significantly fewer accepted any mounts. Aggressive behaviour was not a sexually dimorphic characteristic in this monkey, and passive courtship was also displayed by both sexes.

Normal males displayed masculine copulatory and courtship behaviour in many of their behavioural trials, regardless of whether or not their unfamiliar partner was male or female. However, significantly more males showed mounting and pelvic thrusting behaviour when presented with normal females than androgenized females or other males (Figure 6.7). The time to first mount ranged from 18-782 sec. (0.3-13.0 min.; n=38) but usually occured within 2 minutes. If the mount was not rejected by the partner males did not usually mount again. The duration of the mounts was very variable and lasted from 4 to 185 sec. Males never showed any components of feminine sexual behaviour. Notably, more males threatened and pursued each other than pursued normal females. Otherwise there was no difference in the numbers attacking, submitting or passively courting.

Significantly more normal females displayed feminine courtship and copulatory behaviour to normal males than to normal females (Figure 6.8). Normal females accepted more mounts from normal males than from androgenized females. One female exhibited pursuit courtship to normal and androgenized females, but no other masculine sexual behaviour was observed. In an analagous situation to the males, more females threatened and pursued normal and androgenized females than pursued normal males (Figure 6.8).

Androgenized females exhibited less persistent masculine sexual behaviour to unfamiliar partners of either sex than was observed with normal males (Figure 6.9). The time to first mount ranged from

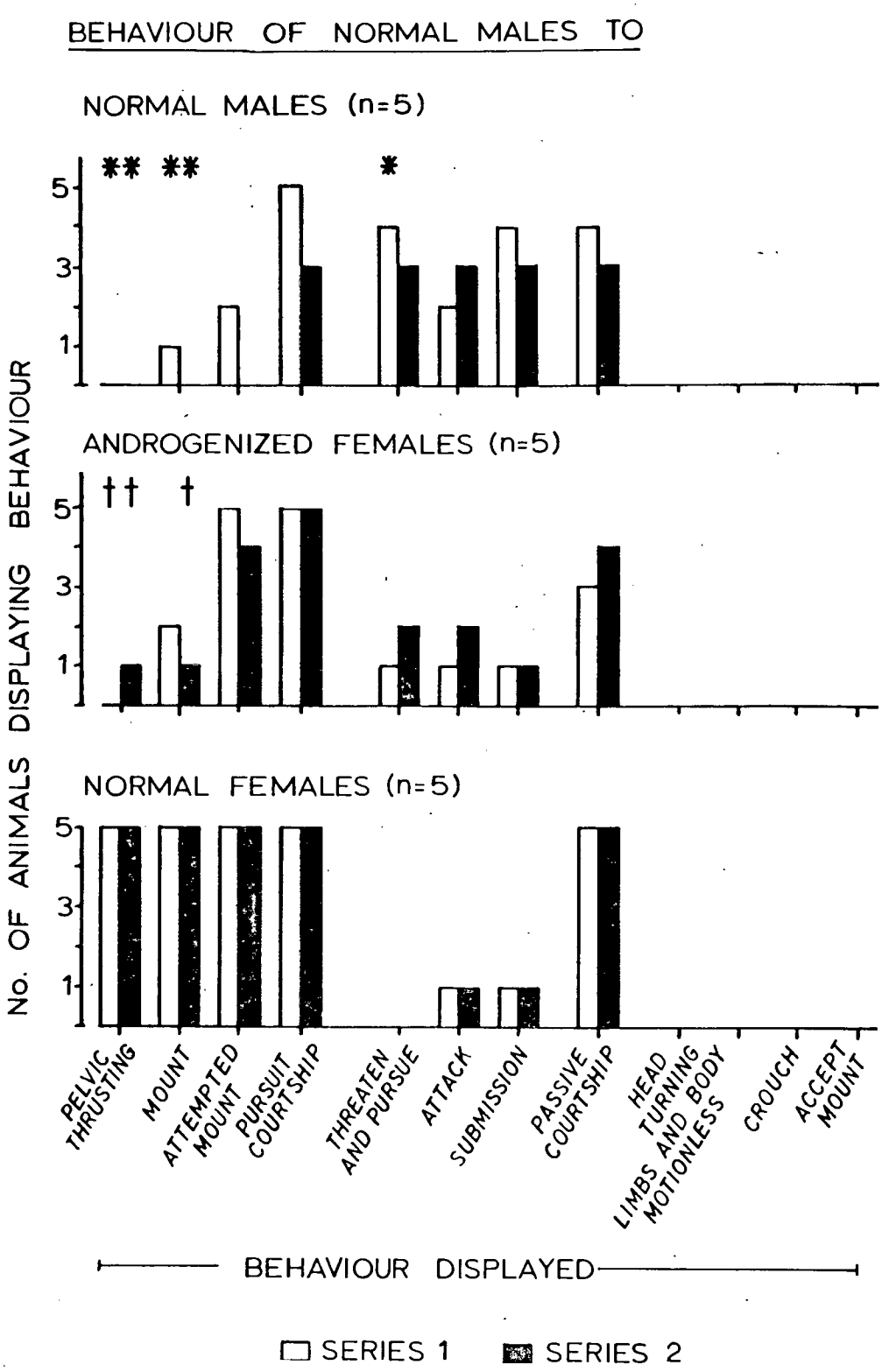


FIGURE 6.7: Behaviour displayed by normal males to other normal males, androgenized females and normal females during 15-min. behavioural trials. Significant differences ($p < 0.05$) were found between males showing behaviour to : (*) other normal males and normal females, and (†) androgenized females and normal females.

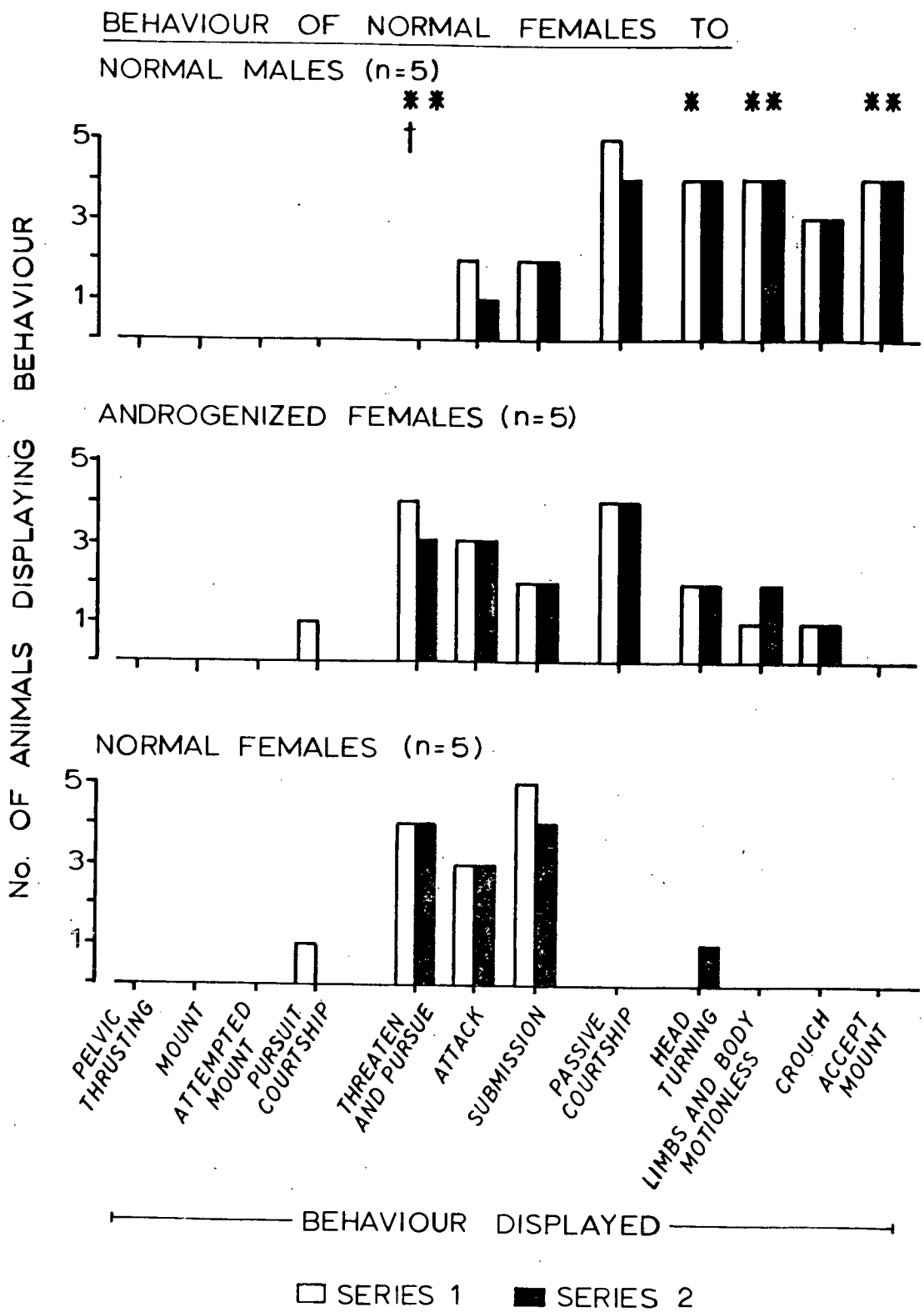


FIGURE 6.8: Behaviour displayed by normal females to other normal females, androgenized females and normal males during 15-min. behavioural trials. Significant differences ($p < 0.05$) were found between females showing behaviour to: (*) other normal females and males, and (†) androgenized females and males.

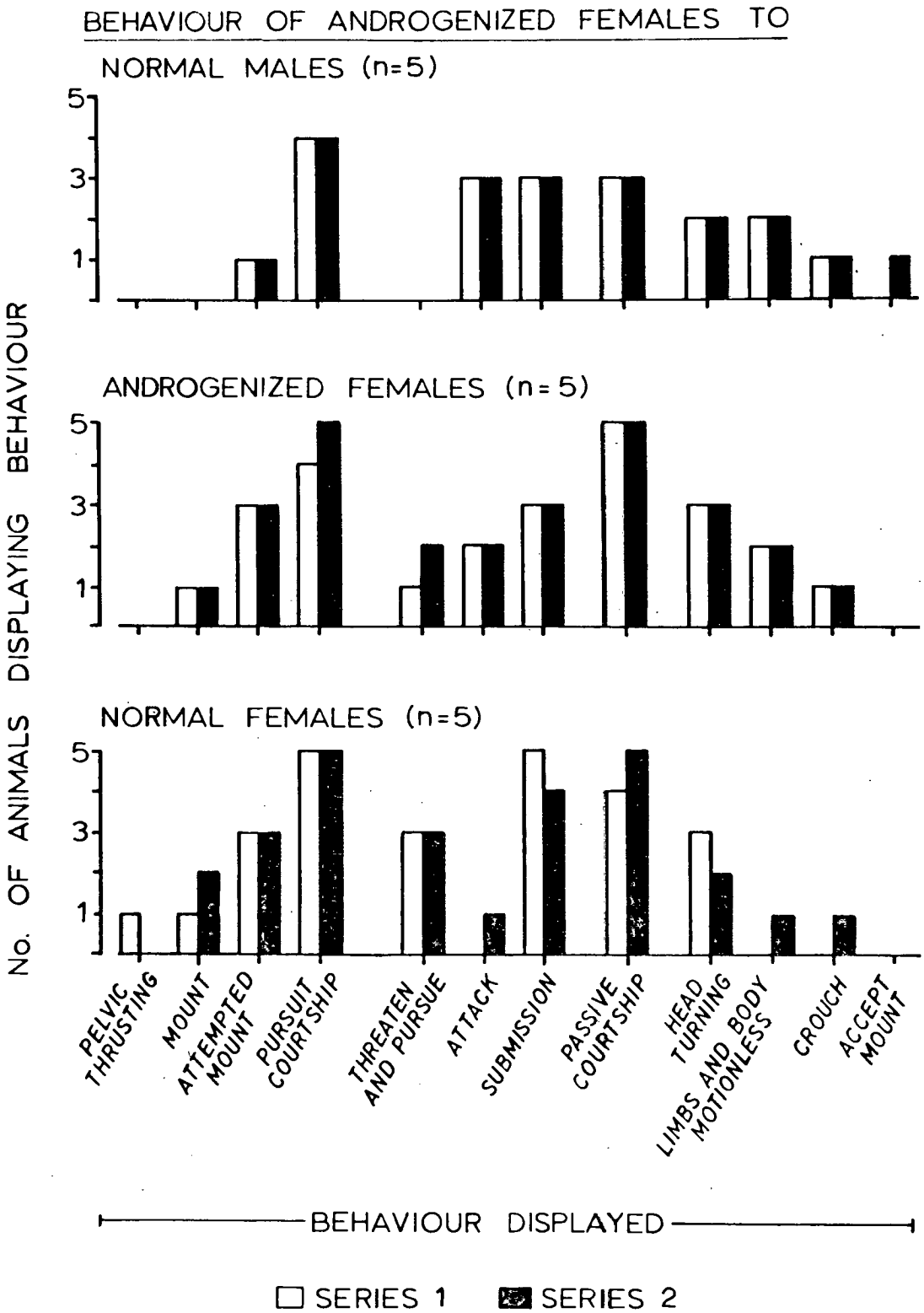


FIGURE 6.9: Behaviour displayed by androgenized females to other androgenized females, and normal males and females in 15-min. behaviour trials.

41-589 sec. (0.7-9.8 min.; n=6), well within the normal male range. However,it was noticeable that androgenized females mounted less frequently than normal males and never mounted as rapidly (41 as opposed to 18 seconds). Similarly to males, if the first mount was not rejected, androgenized females did not mount again. The duration of the mounts was again very variable, ranging from 4-167 secs. However, androgenized females also displayed components of female sexual behaviour to male or female partners. Surprisingly there were no instances where significantly more of these females displayed different behaviour to male, female or androgenized females partners (Figure 6.9).

6.3f (ii) Normal females (born with a male co-twin) and normal females (born with a female co-twin).

Females whether born with a male or female co-twin displayed identical sexual responses (Figure 6.10). All displayed feminine copulatory and courtship behaviour and only one female in each group showed the normally masculine pursuit courtship. No mounting behaviour was observed.

6.3f(iii) Gonadectomized males and females and normal animals

Orchidectomized males did not show any of the pelvic thrusting or mounting behaviour of the intact males (summarized in Figure 6.11). At best, two of them lay across the back of a female, after prolonged sessions of passive courtship (approximately 30 sec.), but made no attempt to climb on. However, all three castrated males did show pursuit courtship to females and two of the females responded with head turning, motionless and crouching behaviour. All three castrated males showed submissive behaviour to one or more intact males (the

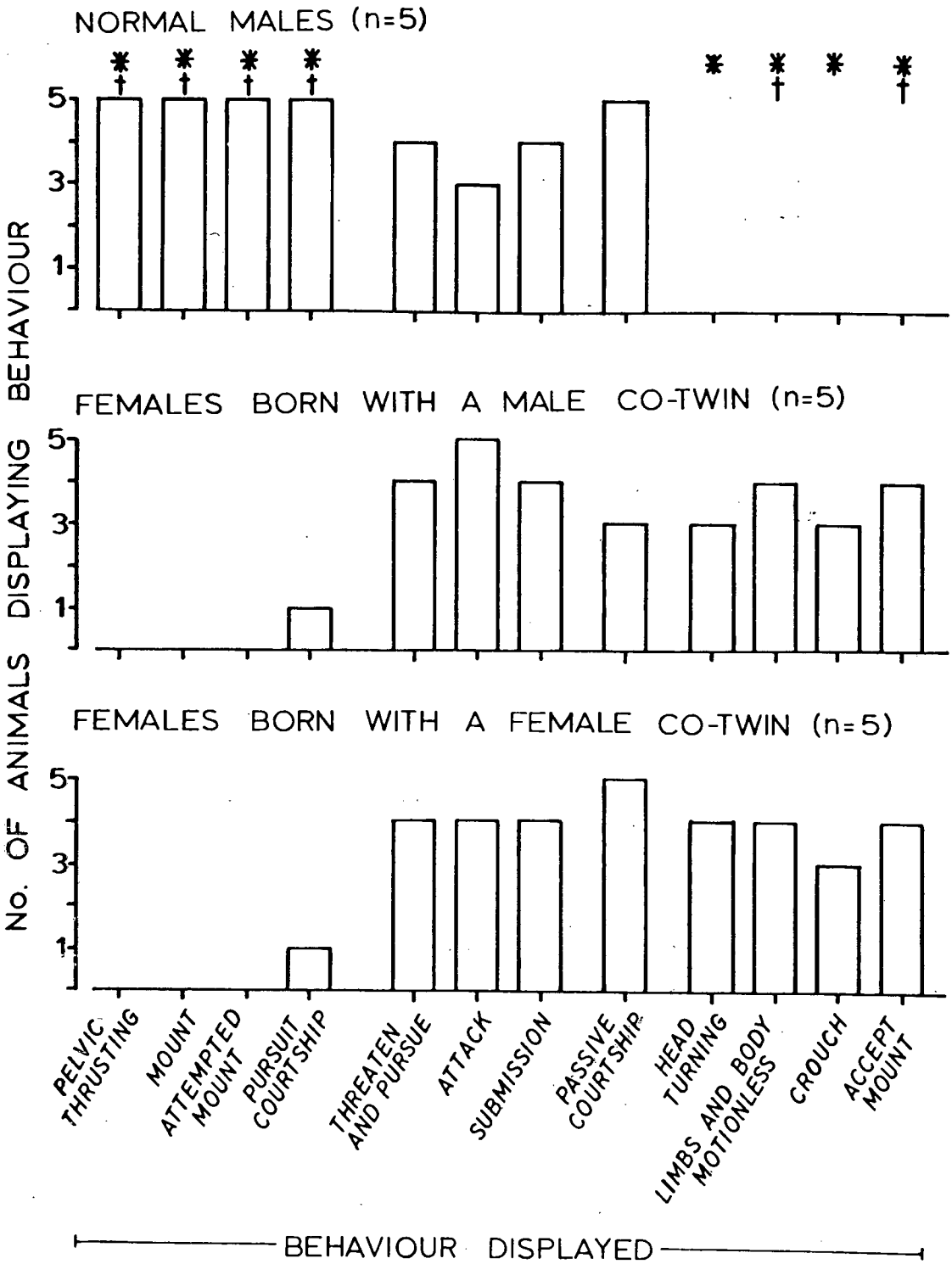


FIGURE 6.10: Masculine and feminine behaviour displayed by normal males, normal females (born with a male co-twin) and normal females (born with a female co-twin) in 15-min. behavioural trials. Significant differences ($p < 0.05$) were found between: (†) normal males and females with a male co-twin and (*) normal males and females with a female co-twin.

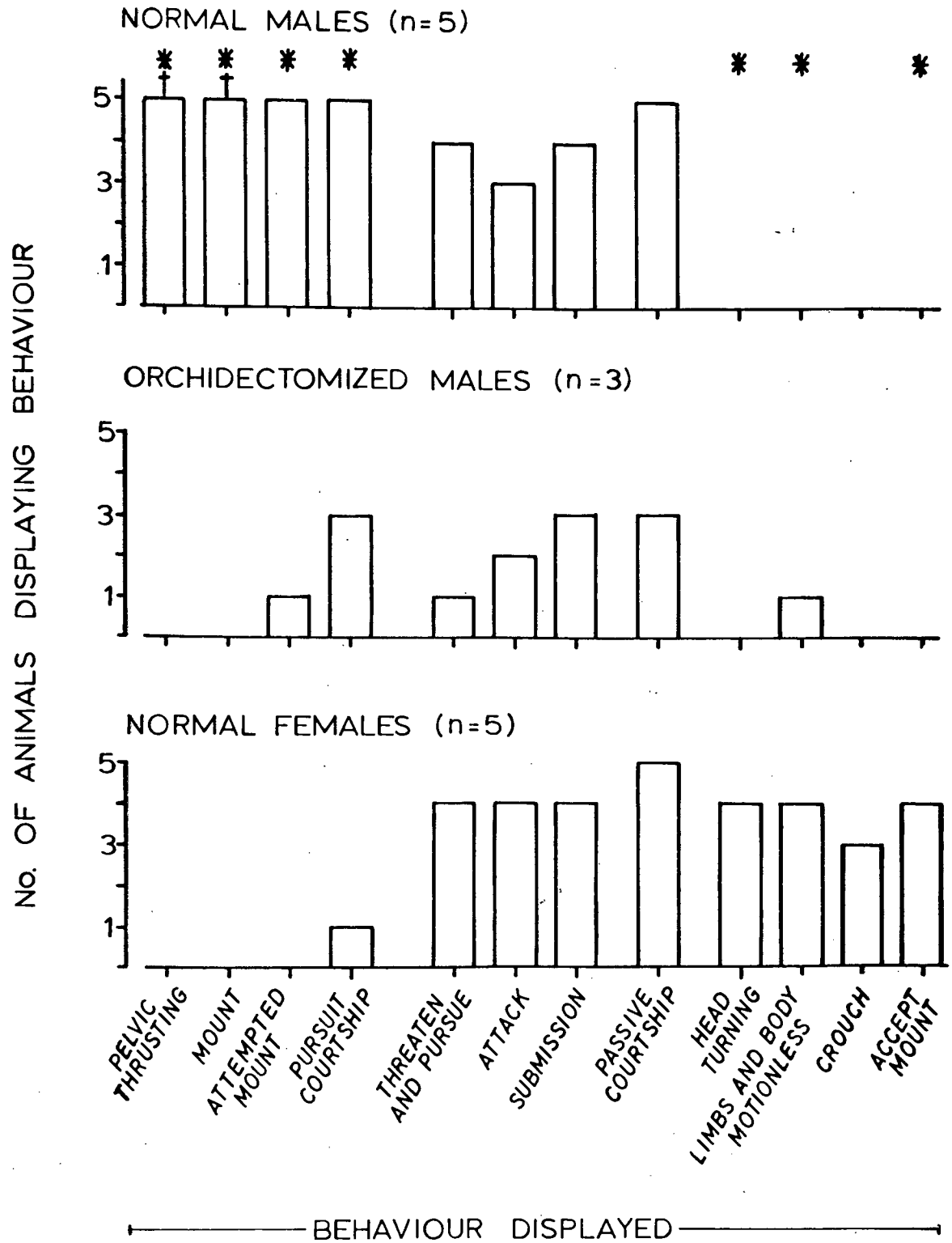


FIGURE 6.11: Masculine and feminine behaviour displayed by normal male, neonatally orchidectomized males and normal female marmosets during 15-min. behavioural trials. Significant differences ($p < 0.05$) were found between: (*) normal males and females, and (†) normal males and orchidectomized males.

converse did not occur) and one castrated male showed the motionless response of the female to an intact male.

Only one out of the three ovariectomized females showed head turning, motionless and crouching behaviour to male partners and none accepted any mounts (summarized in Figure 6.12). As a consequence fewer males mounted ovariectomized females, but this was not significant. One ovariectomized female displayed the pursuit courtship of the male to other females, but no other male sexual behaviour was seen. One ovariectomized female showed submissive behaviour to an intact female.

6.3g The fertility of female marmosets exposed to neonatal testosterone

Between 400 and 600 days of age, four of the androgenized females and two out of three of the remaining control sisters ovulated (as determined by plasma progesterone concentrations of $\geq$ 20ng/ml; see Table 6.6). The progesterone profile during the luteal phase of the cycle was similar to that of their control sisters (Figure 6.13). By 530-730 days of age two of the androgenized females had become pregnant along with both remaining controls (Table 6.7). Three of the pregnancies spontaneously aborted and about half of the offspring in the full-term pregnancies were not weaned, but this was typical of animals of this age (see Chapter 4). There was no apparent inhibition of lactation in androgenized females and one female has raised her offspring quite successfully, From casual observation, there was no noticeable lack of maternal care from androgenized females when they were relatively inexperienced at rearing young.

6.3h The effects of orchidectomy and ovariectomy on the pattern of LH secretion in developing male and female marmosets

In two of the three males orchidectomized by four days of age, and in one of their three sham-operated brothers, there was a small

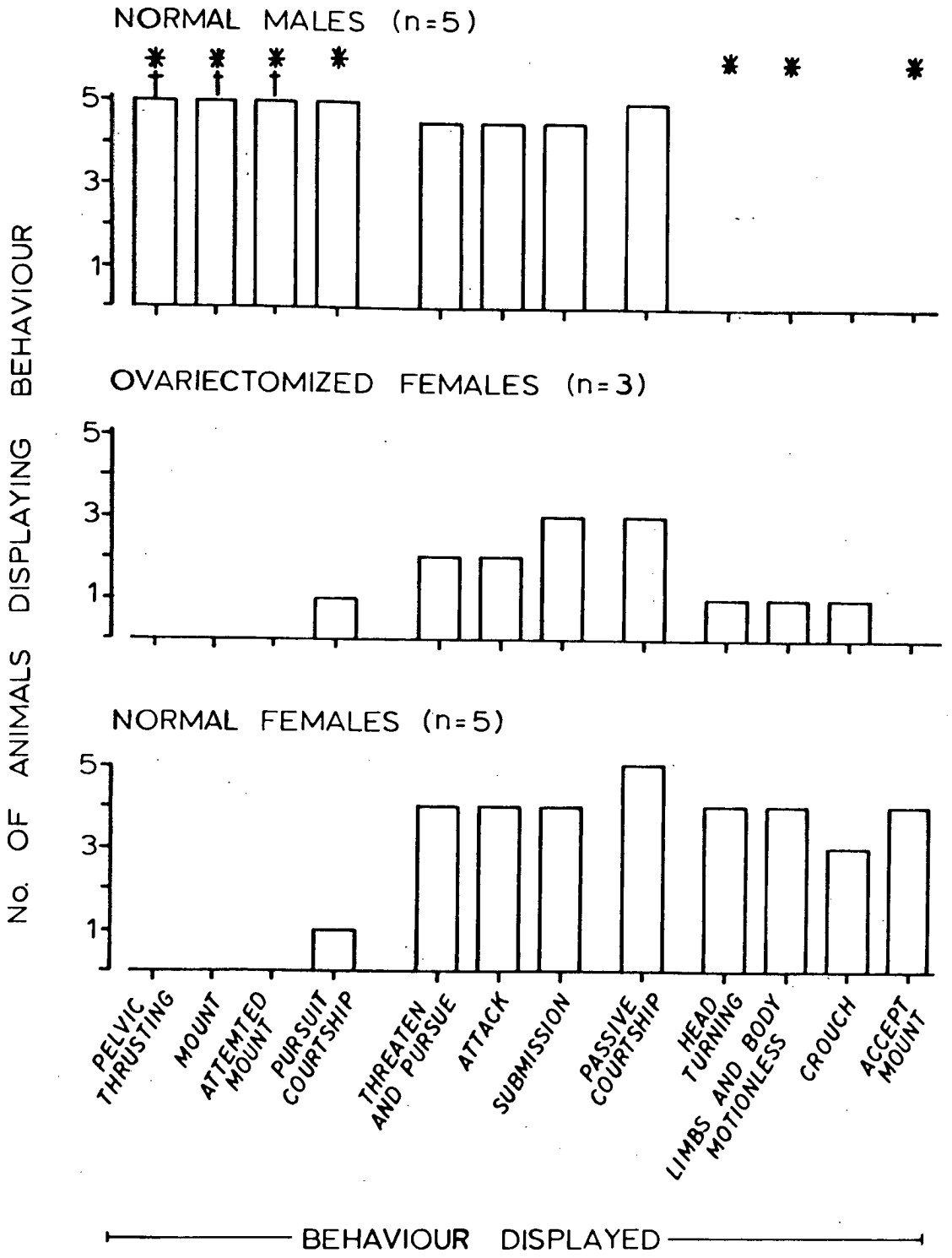


FIGURE 6.12: Masculine and feminine behaviour displayed by normal male, ovariectomized female and normal female marmosets during 15-min. behavioural trials. Significant differences ($p < 0.05$) were found between: (*) normal males and females, and (†) normal males and ovariectomized females.

TABLE 6.6: The age of androgenized and control females when the first luteal phase concentrations of plasma progesterone were detected.

ANDROGENIZED FEMALES			CONTROL FEMALES (SISTERS)		
No.	AGE (DAYS)	PROGESTERONE CONCENTRATION (ng/ml)	No.	AGE (DAYS)	PROGESTERONE CONCENTRATION (ng/ml)
1	-	-	1	-	-
2	436	73.4	2	452	111.7
3	421	71.2	3	-[+]	-
4	550	42.4	4	-[+]	-
5	470	69.1	5	444	54.2

[+] Control sister died before puberty.

FIGURE 6.13: Profiles of plasma progesterone concentrations during the ovarian cycles of two androgenized (o,□) and two control (●,■) female marmosets. Day 0 is taken as the first day of the progesterone rise above 20 ng/ml (ovulation occurring 1-3 days previously, P.L. Chambers, personal communication).

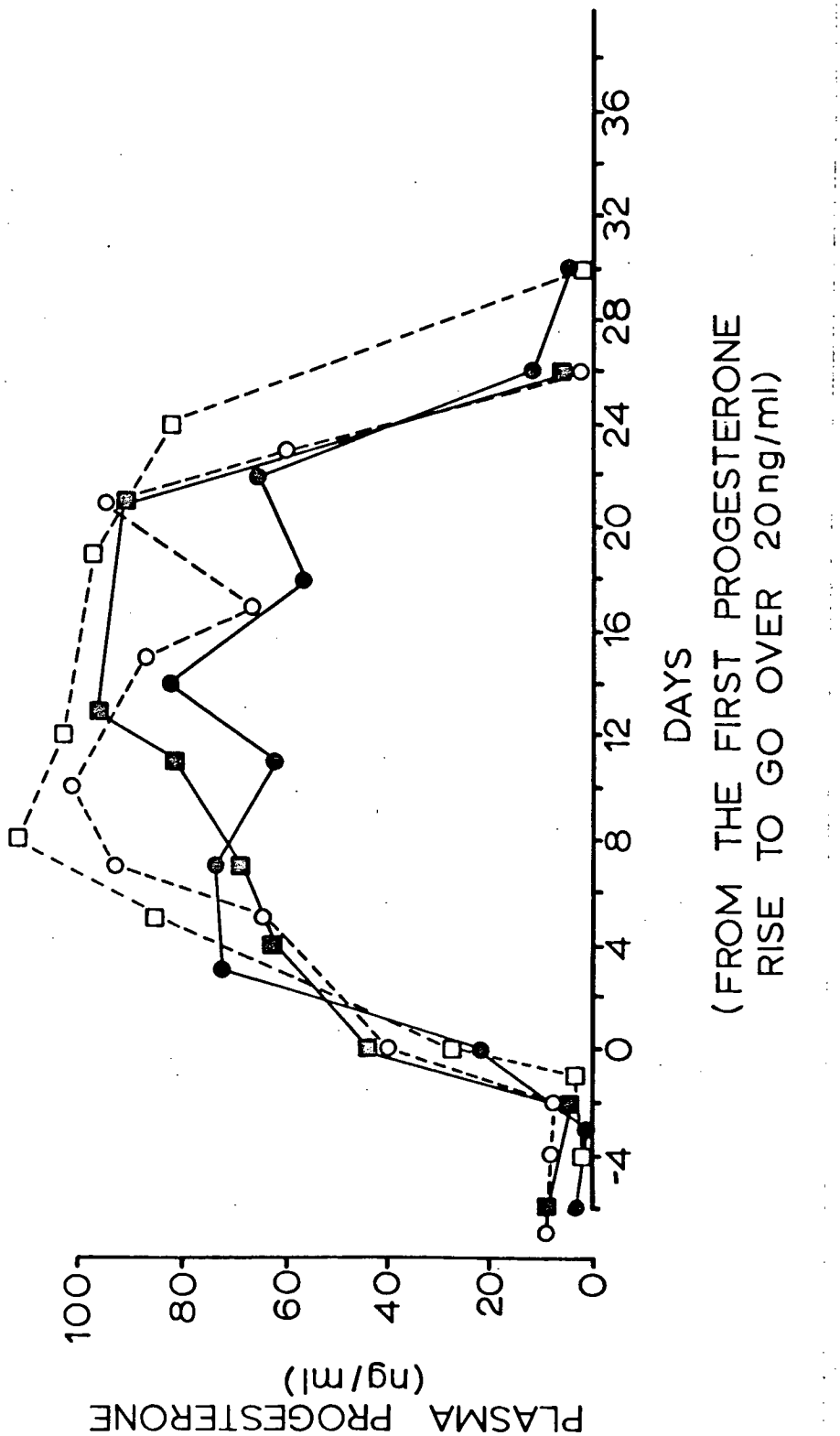


TABLE 6.7: Data pertaining to the first and second pregnancies of androgenized females and their sister controls.

NO.	AGE AT FIRST PREGNANCY (DAYS)	OUTCOME OF FIRST PREGNANCY	AGE AT SECOND PREGNANCY (DAYS)	OUTCOME OF SECOND PREGNANCY
(a) ANDROGENIZED FEMALES				
1	736	Triplets (died)	924	(At mid-gestation)
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	654	Spontaneous Abortion	715	Twins
(b) CONTROL FEMALES				
1	687	Spontaneous abortion	703	Twins (1 died)
2	530	Spontaneous abortion	641	Twins

neonatal rise in the plasma concentration of LH (at about 40-50 days of age; Figure 6.14). LH concentrations then became unmeasurable ($\geqslant$ 20 ng/ml) until the animals reached about 160 days of age, when LH concentrations rose again in two of the castrated animals. The third castrated male was not sampled until 230 days of age, but he also had measureable LH levels at this stage. High levels of LH were maintained in all castrated males thereafter. In the intact control animals of 160 days of age and over, LH concentrations remained at or below the sensitivity limit of the assay. Testosterone concentrations in the castrated males were barely detectable throughout (about 1 ng/ml) and none showed a neonatal rise (Figure 6.15).

Females ovariectomized 1-4 days after birth took much longer than orchidectomized males to show the castration response of elevated LH concentrations (unfortunately none were sampled neonatally). In three of the four ovariectomized females, plasma LH levels did not rise until at least 400 days of age (Figure 6.16), about the age of first ovulation. In the fourth, ovariectomized at 180 days old, LH levels rose at 357 days. LH concentrations in the two intact sister controls remained immeasurable throughout. Plasma oestradiol and progesterone concentrations in ovariectomized females never exceeded 0.4 ng/ml and 6.0 ng/ml, respectively.

6.4 Discussion

6.4a The consequences of post-natal differentiation in primates and Man

This is the first report of an effect of neonatal androgenization on behaviour in a primate. Female rhesus monkeys, treated with

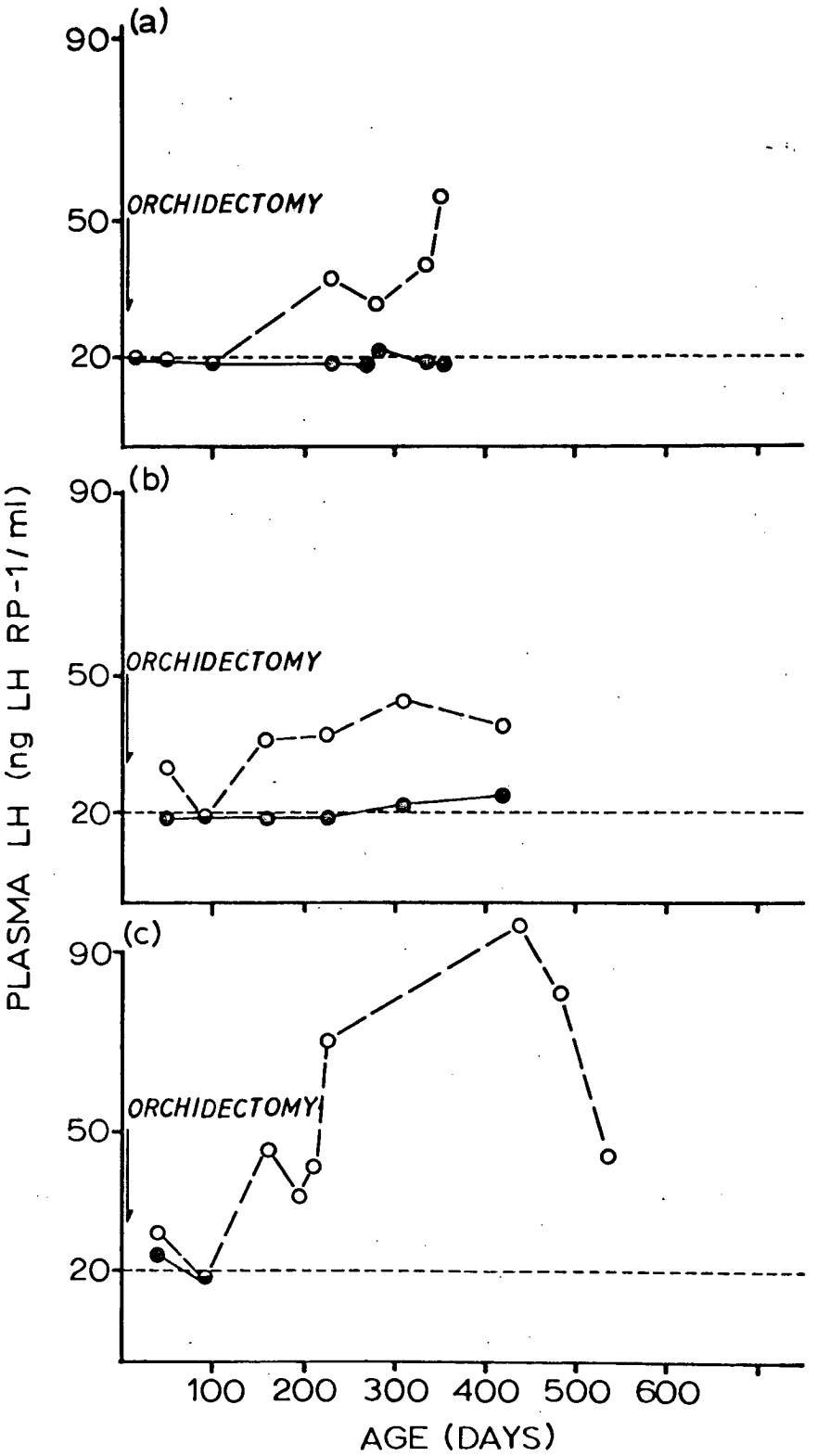


FIGURE 6.14: The concentrations of plasma LH in male marmosets castrated shortly after birth.

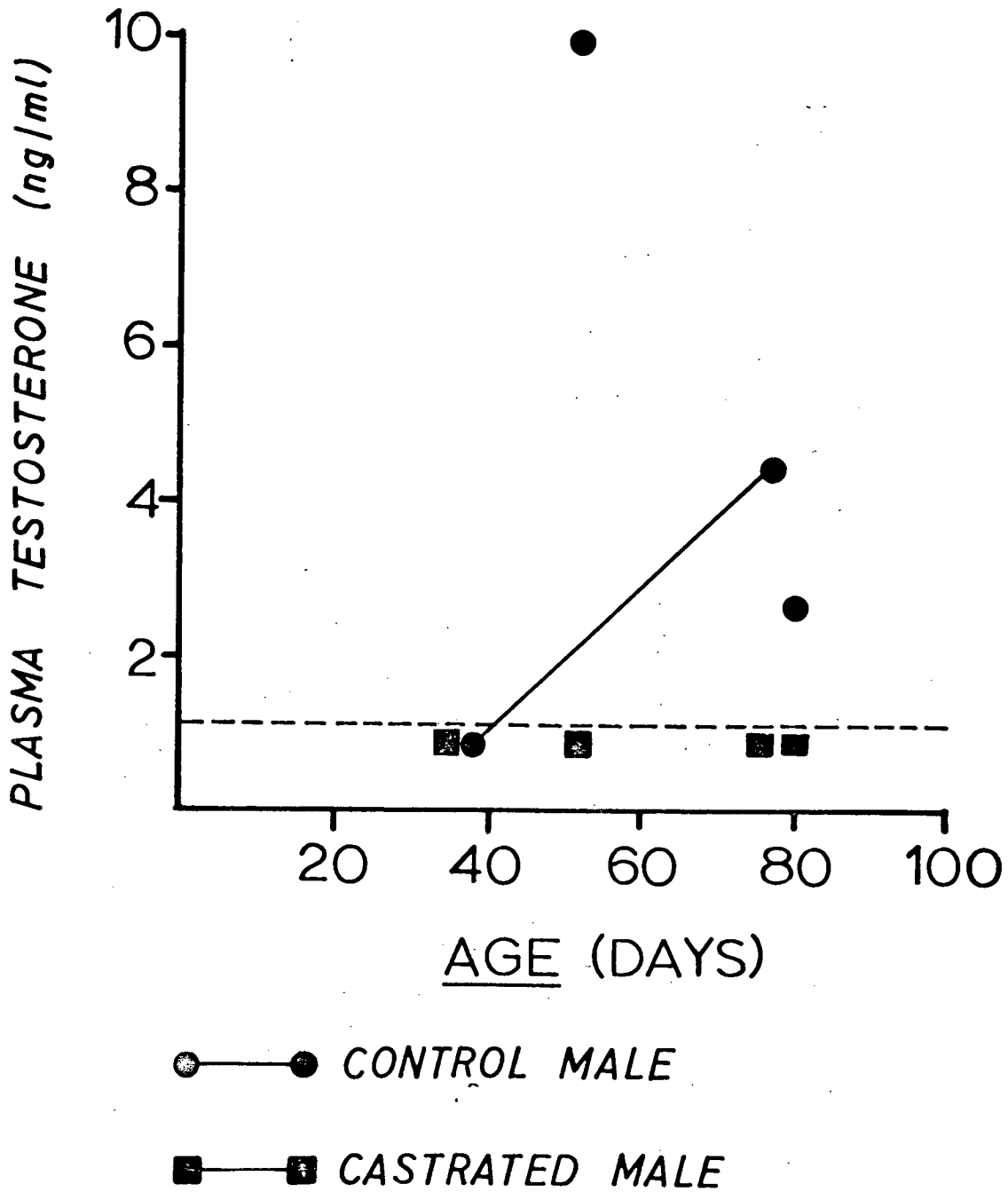


FIGURE 6.15: The concentration of plasma testosterone in neonatal male marmosets castrated shortly after birth and their sham-operated controls.

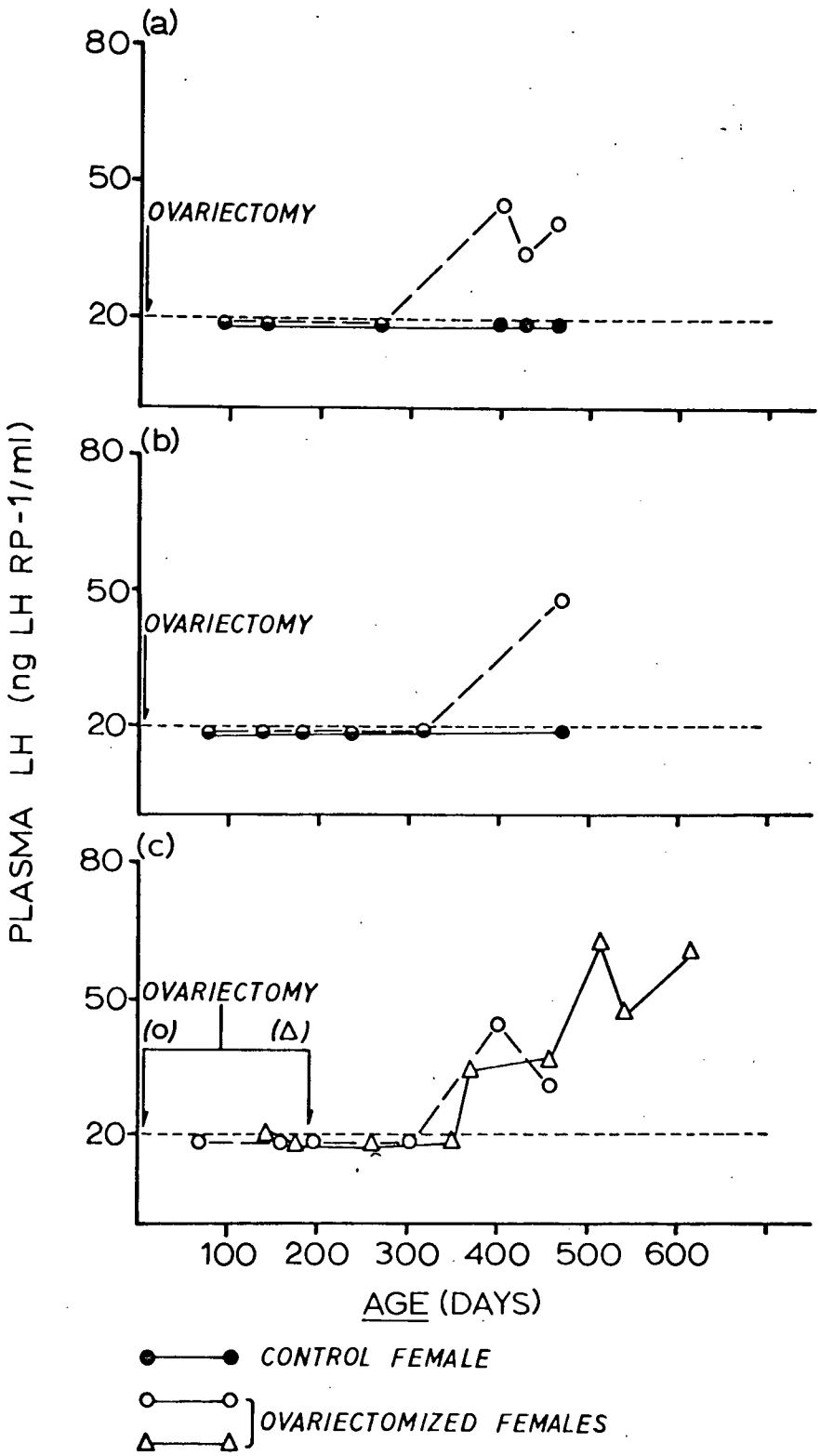


FIGURE 6.16: The concentration of plasma LH in female marmosets ovariectomized shortly after birth and their sham-operated controls.

testosterone propionate between 6.5 and 14.5 months of age, failed to show any signs of behavioural masculinization (Joslyn,1973), and only females treated with androgen before birth developed masculinized behaviour (Goy et al, 1977; Goy, 1978; see Chapter 1). The newborn male rhesus and pig-tailed macaques (Robinson and Bridson, 1978) and the human (Forest et al, 1973a,b; 1974a,b; 1975; 1976; Forest, 1975, in press) all experience elevated concentrations of plasma testosterone, but this period of development was not tested in the rhesus monkey experiments (no females of less than 6.5 months old were implanted with testosterone and observed in later life; Joslyn, 1973). Whether all these primates undergo some sexual differentiation in the immediate postnatal period, or whether the marmoset is unique in this respect, remains to be demonstrated. There is some evidence in rhesus monkeys of dimorphic brain development in the early neonatal period. In males given bilateral brain lesions in the orbitofrontal area soon after birth, the cognifitive deficits associated with the lesion appear at $2\frac{1}{2}$ months of age in learning tests (Goldman, Cranford, Stokes, Galkin and Rosvold, 1974). In females, comparable deficits do not appear until 15-18 months of age. If androgen is given to such females postnatally, they suffer cognitive deficits from this lesion at as early an age as males (P.S. Goldman cited by R.W. Goy, personal communication).

Postnatal differentiation is a particularly important consideration when advocating administering testosterone either to premature babies in the hope of reducing their mortality rate (e.g. Polishuk and Anteby, 1971) or to mothers in labour in order to reduce "after pains" or suppress lactation (British Pharmaceutical Codex 1968; p. 827). Such treatment of newborn or nearly born baby girls might lead to

undesirable behavioural consequences after puberty. Three women who received testosterone treatment neonatally (between 2 and 30 days post-partum) have been re-examined at 20 years of age (Polishuk and Anteby, 1971). All three menstruated and had developed normal secondary sexual characteristics. Two were pregnant. No behavioural observations were reported, but as the testosterone was administered for a relatively short time, behaviour might have been unaffected. Premature boys are known to have elevated levels of circulating testosterone following birth similar to males in full-term deliveries (Forest et al, 1974a). Testosterone levels in premature boys take about a week longer to rise than in full-term deliveries (about 14 days post-partum instead of 7) and are elevated for a longer time, 90-150 days instead of 90-120 days.

Neonatal androgenization of a primate like the marmoset also has advantages over the methods of fetal androgenization used in rhesus monkeys (e.g. Goy et al, 1977; Goy, 1978). In the marmoset, the exact dose of testosterone the animal receives is known. In the rhesus, only the dose given to the mother is known. The exact amounts of androgen crossing the placenta to the fetus are not. There is also no wastage of animals from neonatal treatment while a considerable number of pregnancies are lost when fetal androgenization is attempted (e.g. rhesus monkeys: Goy et al, 1977; marmosets: J.P. Hearn, personal communication). The dosage of testosterone given to neonatal female marmosets was similar to that used to masculinize other female mammals (Table 6.8).

6.4b The effects of androgenization on growth, sexual development and fertility in female primates

Since there is no sex dimorphism in body size in marmosets (Chapter 3), it was not surprising to find no effect of neonatal

TABLE 6.8: A comparison of androgen doses given to androgenize female fetuses or neonates of different mammalian species.

ADMINISTERED TO:	ANIMAL	ESTIMATED DOSE (mg/kgBW/day)		REFERENCE
Mother	Rhesus monkey	2.5	TP	Phoenix et al, 1968
	Dog	1.1	TP	Beach and Kuehn, 1970
	Guinea pig	6.0	TP	Brown-Grant and Sherwood, 1971
	Sheep	0.1	T	Clarke, 1976
Neonate	Marmoset	3-10	T	Present study
	Rat	125	TP*	Barraclough, 1968
	Hamster	30	TP*	Swanson, 1971

* = Single injection

BW = Body weight

TP = Testosterone propionate

T = Testosterone

androgenization on the body weight of female marmosets (Table 6.1). In contrast, fetally androgenized female rhesus monkeys begin to develop some of the males' larger size by increasing their body weight (Wilen et al, 1977). It was also not surprising that neonatal testosterone did not masculinize the female marmosets' external genitalia as they were fully differentiated at birth. The testosterone treatment, however, induced permanent hypertrophy of the clitoris (Figure 6.3) as found in rhesus females given testosterone neonatally (Joslyn, 1973).

Androgenized female rhesus monkeys showed delayed menarche (Goy and Resko, 1972; Wilen et al, 1977), and menstrual bleeding occurred through the "penis" of their masculinized genitalia (Phoenix, 1974). Delayed menarche was also found in girls androgenized in utero (Jones and Verkauf, 1971). It was not possible to ascertain whether androgenization delayed puberty in female marmosets, as this would have required serial determinations of progesterone throughout the pubertal period. However, the animals did start ovulating at the normal age (Table 6.6) and seemed to exhibit normal cycles (Figure 6.13). Hence, in agreement with the results from androgenized fetal, or neonatal female rhesus monkeys (Goy and Resko, 1972; Treloar et al, 1972) and human females exposed to testosterone in utero or neonatally (Wilkins et al, 1955; Polishuk and Anteby, 1971), androgenized female marmosets did not develop the anovulatory syndrome in later life. In the marmoset this was understandable since intact adult males and females retain the positive feedback response of LH to oestradiol administration (Hodges and Hearn, 1978). Androgenization also did not affect their fertility as two of the experimental animals became pregnant as well as both of their remaining control sisters (Table 6.7).

Both groups have raised offspring, showing there was no inhibition of lactation. Women androgenized during fetal life have also raised their own offspring (Money and Ehrhardt, 1972) and women who were given testosterone neonatally have become pregnant (Polishuk and Anteby, 1971).

6.4c The effects of androgenization on female playful and aggressive behaviour and the dimorphic characters of these behaviours

The pre-pubertal rough-and-tumble play of androgenized female marmosets in female-female twin pairs was enhanced to an extent normally found in males in male-female twin pairs (Table 6.3), suggesting that neonatal exposure to testosterone had had some organizing effect on female behaviour. Nevertheless neonatally castrated males in male-male twin sets did not initiate less rough-and-tumble play than their intact brothers, suggesting that some prenatal organisation of behaviour had occurred in these males because they did not show a 'feminine' score against a brother (Table 6.3). The fact that the normal male shows more of the aggressive elements in play than the female has been previously found in pubertal male marmosets (Abbott, 1978) and in many other male primates, such as rhesus monkeys (Mason, 1960; Harlow and Harlow, 1965; Goy and Resko, 1972; Brandt and Mitchell, 1973), crab-eating macaques and patas monkeys (M. fascicularus and Erythrocebus patas; Seay, Schlottman and Gandolfo, 1972), talapoin monkeys (M. talapoin; Wolfheim, 1977), baboons (Papio sp.; DeVore, 1963; Ransom and Rowell, 1972), squirrel monkeys (S. sciureus; Baldwin, 1969), vervet monkeys (C. aethiops; Lancaster, 1971), chimpanzees and orang-utans (P. troglodytes and Pongo pygmaeus; Nadler and Braggio, 1974), and the human (Davenport, 1965; Brindley, Clarke, Hutt, Robinson and Wethli, 1973;

Blurton-Jones and Konner, 1973; Braggio, Nadler, Lance and Miseyko, 1978). However, in marmoset play sex differences were limited to rough-and-tumble and were not as prevalent throughout all the different types of play behaviour as in the other primates listed above. Stevenson and Poole (1976) and Stevenson (in press, b) have reported no sex differences in marmoset play. This anomaly probably arises from the different modes of analysis, the latter authors analysing sequences of play behaviour and initiation of complete play bouts, while in this study only the frequency of each play event was analysed.

It has been suggested that aggressive play characteristics in young males are related to their aggressiveness and dominance when adult (Gray, 1971; Nadler and Braggio, 1974). However, not all primate species have males as either the highest ranking group members or as the most aggressive, such as among talapoin monkeys (Dixson et al, 1973; Wolfheim, 1977), vervet monkeys (Rowell, 1972), squirrel monkeys (Baldwin, 1969) and marmosets (Ingram, 1975; see Chapter 5 and Sections 6.3e and f). Nevertheless, play differences have been observed between young males and females in these species and, as Wolfheim (1977) suggests, the tendency of young males to initiate more aggressive play than females is not necessarily a precursor of adult male behaviour. In the marmoset, from the behaviour of males and females in peer groups and behavioural trials, aggression is not a sexually dimorphic trait, and this is perhaps related to monogamy. In the polygamous rhesus monkey, males and androgenized females displayed more aggression than normal females (Goy and Resko, 1972; Eaton, Goy and Phoenix, 1973; Goy, 1978). Interestingly, female rhesus monkeys given testosterone propionate neonatally gained in dominance status among their peers, and during that change in status they also showed increased aggressive behaviour (Joslyn, 1973). Unlike the prenatally

androgenized rhesus females, play was not affected. Thus, in female rhesus monkeys, postnatal androgens may augment social assertiveness and aggression without concomitantly augmenting masculine forms of play (or sexual behaviour).

It was interesting that in the behavioural trials male marmosets threatened and pursued other males more than females and that females threatened and pursued other females more than males (Figures 6.7 and 6.8). This pattern of aggression was identical to that which predominated in peer groups of males and females (Chapter 5). In contrast, androgenized females did not show any preference for threatening and pursuing normal males, normal females or other androgenized females (Figure 6.9) and they were threatened and pursued by neither normal males or females in particular (Figures 6.7 and 6.8). This may have been due to their ambiguous sexual responses, or perhaps the ambiguous appearance of their external genitalia.

Castrated male marmosets never became dominant in any peer group, but half of the ovariectomized females did (section 6.3c), suggesting that androgens are important in males for achieving dominance status whereas ovarian hormones are not important for achieving such status in females. This result was reinforced in the behavioural trials when all the castrated males showed submissive behaviour to one or more intact males but this was not found when ovariectomized females faced intact females. In the case of the males, this data contrasts with that obtained from intact males when there was no significant difference in plasma testosterone levels between the dominant and subordinate males in a group (Chapter 5). However, chronically low testosterone from birth might be the important factor leading to the subordinate status of castrated males. Whether this is a maturation effect remains

to be determined. Neonatal castration of male rhesus monkeys did not always lead to their low status among males in groups (Bielert, 1978). In one of the three groups studied when the monkeys were over a year old, low ranking castrated males given testosterone propionate shifted from dominance positions 4 and 6 to positions 1 and 3, respectively. In another group the castrates already occupied high social status and testosterone failed to alter their rank. Apparently, exogenous testosterone can elevate the status of low ranking castrated males, but if the castrated males already hold high rank testosterone is not effective. In the case of the females, the seeming unimportance of ovarian function in achieving dominance status deepens the mystery of what qualities females possess to become dominant. The previous results (Chapter 5) suggested that cyclic ovarian function might be an important factor in attaining dominance, but the above results tend to contradict this. The ways in which females attain dominant status require further investigation.

6.4d The effects of androgenization on female sexual behaviour and the consequences for postnatal differentiation in the marmoset

In the behavioural trials, the androgenized female marmosets exhibited masculinized sexual behaviour, though not to the degree observed in normal males (Figure 6.9). Whether this is due to their lack of 'persistence' in attending to normal females remains to be shown (see Andrew, 1978 for review). In addition, the androgenized females also showed normal female sexual responses (in the peer groups and behavioural trials), indicating that masculinization of their sexual behaviour could occur without defeminization. This result is in keeping with work on other species such as rats (Clemens et al, 1969), hamsters (Payne, 1976) and dogs (Beach and Kuehn, 1970; Beach et al, 1972)

where androgenized females also showed enhanced masculine behaviour without the loss of their feminine sexual responses. Normal male marmosets displayed no feminine sexual behaviour and, apart from one female in each female group showing the masculine pursuit courtship, no female displayed masculine sexual behaviour (Figures 6.6, 6.10, 6.11 and 6.12).

These findings suggest three possible mechanisms for marmoset sexual differentiation and development. Firstly, because androgenized females show both masculine and feminine sexual behaviour and males only show masculine behaviour, this suggests that an 'event' has occurred during the development of the normal male, that does not happen in the androgenized females, to prevent males showing feminine behaviour. A similar conclusion was reached from the rough-and-tumble play results. In other words, perhaps male fetuses receive androgens prenatally as well as postnatally (as is the case with rhesus monkeys and Man), and their female co-twins are in some way 'protected' against the virilizing actions of these hormones prenatally (perhaps by insensitivity to androgens or by having a different 'critical period' for sexual differentiation to the male; see Chapter 7 for further discussion). This possibility implies that marmosets undergo both prenatal and postnatal differentiation. In further support of prenatal differentiation of behaviour, male marmosets castrated at birth showed some components of masculine sexual behaviour, but little or no feminine behaviour (Figure 6.11). So apparently males are at least partly masculinized before birth. However, the already differentiated external genitalia of the neonatal castrates confounds this point, because other animals may treat them as male because of their appearance and hence bias the castrates' behavioural responses (male and female

marmoset infants are treated differently by their parents and siblings; Ingram, 1977). Whether the above difference found in this study reflects different periods of behavioural differentiation between the sexes, or pre- and postnatal 'critical periods' in both sexes remains to be seen. The reduced masculine behaviour of the castrated males, especially the absence of pelvic thrusting and normal mounting behaviour, may be due to the lack of postnatal androgens, pubertal and post-pubertal androgens, or a combination of all three. Juvenile male rhesus monkeys castrated at birth displayed similar frequencies of mounting behaviour to intact juvenile males, demonstrating that after birth testicular androgens were not essential for the development and display of this behaviour in that species (Goy et al, 1977; Goy, 1978). However, in adulthood, neonatal castration could lead to reduced libido in some animals (R.W. Goy, personal communication).

A second possible mechanism for differentiation arises because the androgenized female marmosets were tested for behaviour in post-pubertal and adult life under the influence of their own ovarian hormones (Table 6.6) and without the testicular testosterone levels of the normal male controls. Hence, this hormonal difference may mediate the difference in behaviour between androgenized females and normal males. This latter possibility is compatible with the hypothesis that there is no sexual differentiation of the marmoset brain in fetal life and that it is entirely postnatal, with an initial organizing effect produced by an early postnatal surge of testosterone, and then a later reinforcing effect with rising testosterone levels at the onset of puberty. In further support of this hypothesis, fetal male marmosets have similar circulating testosterone levels to their co-twin females (less than 1.1 - 2 or 3 ng/ml; P.L. Chambers, personal communication)

implying that there is no difference in the hormonal 'imprinting' of either sex before birth. Unfortunately, the earliest time fetuses were sampled was at 80-90 days of gestation (in the latter half of pregnancy). By this time the difference in hormone levels between the sexes may have disappeared. In the human, the early sex differences in circulating testosterone levels either disappears (Forest, Ances, Tapper and Migeon, 1971; Reyes et al, 1974) or is of small magnitude (Forest et al, 1973b) in late gestation, but in rhesus monkeys the difference is maintained throughout pregnancy (Resko, 1977). However, it is unlikely that if there is a fetal testosterone surge in the male marmoset it can dissipate by 80 - 90 days of gestation, because the gonads do not differentiate until about 70 days (mid-pregnancy; W-S. O., P.L. Chambers, J.P. Hearn, personal communication). Therefore, because fetal testosterone levels are similar in both sexes, either there is no fetal male testosterone surge in the marmoset or females co-twinned to a male experience his testosterone levels. Hence the evidence for hormonally induced prenatal differentiation of the marmoset brain is equivocal, but this does not mean that marmosets undergo differentiation in a similar way to rats and mice (see Chapter 1).

The relatively late development of marmoset gonads, in comparison with the rhesus monkey (Van Wagenen and Simpson, 1965) and the human (Moore, 1973), may be due to the overall slow pace of marmoset embryogenesis during the first half of pregnancy (Phillips, 1976b). This, in turn, may be due to an adaptation to the normally high energy drain on pregnant marmosets which are still lactating. There is no lactational amenorrhea in this monkey and females frequently become pregnant within 1-3 weeks of giving birth (P.L. Chambers and J.P. Hearn, personal communication). But, their infants are not weaned until about

40 days post-partum and they still suckle sporadically up until 80-90 days (Hearn et al, 1975). A similar reproductive timetable seems to exist in the wild (Stevenson, in press, a). After about mid-pregnancy marmoset fetal development continues apace (Phillips, 1976b) and the placenta undergoes massive growth (P.L. Chambers and J.P. Hearn, personal communication).

Attempts have been made to androgenize female marmosets in utero by implanting pregnant animals with 25 or 50 mg. of testosterone, but so far all the pregnancies have spontaneously aborted and the fetuses lost (J.P. Hearn, personal communication). This hazard has also been encountered while attempting to androgenise fetal female rhesus monkeys (Goy et al, 1977). Phillips (1975) has given three pregnant marmosets 0.3mg. of a testosterone compound (Primoteston; Schering Chemicals) once every two weeks for a total of fourteen weeks (approximately 1 mg/kg. maternal body weight per dose). The stage of pregnancy was not reported, and when five fetuses were recovered after hysterectomy (again no age reported), no "abnormalities" were found, but the genetic sex of the animals was not given. The frequency of dosing was also low in comparison with the daily dosing of rhesus monkeys (Goy et al, 1977). Whether the female marmoset can be masculinized in utero still requires clarification.

Finally, the differences in sexual responses between normal male and female marmosets and androgenized females may not be exclusively due to the behavioural organizing effect of neonatal testosterone. The neonatal testosterone may have simply altered the hormonal state of the androgenized females by affecting ovarian, hypothalamic, pituitary or adrenal function, which in turn could be partly or wholly responsible for their altered behaviour. However, in at least one respect the ovarian function of androgenized female marmosets appears normal

(Table 6.6, Figure 6.13). Nevertheless, the behavioural responses of normal adult male and female marmosets to exogenous testosterone or oestradiol have not yet been tested. It will be important to establish whether or not masculine sexual behaviour is enhanced in normal females just by exposure to testosterone in adulthood, and whether or not feminine sexual behaviour is enhanced in normal adult males exposed to oestradiol. The animals would have to be gonadectomized before the hormones were administered to rule out any endogenous hormonal effects. If these treatments did not reverse the sexual behaviour of either sex, then the behavioural repetoire of marmoset monkeys must be 'masculinized' or 'feminized' during development.

Female marmosets ovariectomized at birth showed little or no feminine sexual behaviour in the behavioural trials (Figure 6.12), suggesting that ovarian hormones might be necessary for the display of these behaviours and to maintain female receptivity (i.e. 'willingness' to mate). Nevertheless, the two dominant ovariectomized females displayed normal copulatory and courtship behaviour in their peer groups. As the pattern of <u>feminine</u> sexual behaviour was identical to that of the intact androgenized females, perhaps the postnatal testosterone has suppressed the feminine behaviour of the latter to some degree and made them less responsive to the advances of the male. A similar situation has been found in androgenized ewes where prenatal testosterone had the effect of decreasing the females' potential to show feminine behaviour (Clarke, 1977). Prenatal testosterone also enhanced their potential to show masculine behaviour, but this was viewed as a separate process (see Chapter 1).

The predominance of copulatory responses from normal male marmosets to many of their partners (regardless of sex) added interest to the behavioural trials (Figure 6.7). Because of the lack of

dimorphism in this monkey, perhaps males 'mistake' other males for females or perhaps they just attempt to copulate with any strange animal until they are rejected, irrespective of whether or not it is trying to copulate with them. Androgenized females showed similar behaviour (Figure 6.9).

6.4e The effects of gonadectomy on the LH secretion of neonatal males

Two out of the three male marmosets castrated at birth showed a neonatal rise in circulating LH concentrations (Figure 6.14) at a time when males normally experience high testosterone levels. Apparently their hypothalamic-pituitary negative feedback response was sensitive to testicular androgens at this time. This indicated that whether or not much of the circulating testosterone was bound to a sex hormone binding protein (as is the case in humans; Forest et al, 1974a) it was having a biological effect at least at the negative feedback level. Normal males also showed elevated LH levels accompanying their neonatally elevated testosterone levels (Figures 6.14 and 6.15; Chapter 4) and a similar thing happens in newborn boys (Faiman et al, 1974). Oddly enough, following the neonatal rise of LH in castrated male marmosets, LH levels became immeasureable (typical of intact pre-pubertal animals) until about 160 days of age, even though the animals had no testes (Figure 6.14). Their pituitaries had apparently 'switched off'. A similar result was found when male rhesus monkeys were castrated at birth (R.W. Goy, personal communication). Neonatally, the concentration of circulating gonadotrophins increased greatly and at a later time in maturation they fell back to concentrations typical of normal pre-pubertal males until puberty. What the neonatal androgen is accomplishing in rhesus monkeys in terms of physiological

differentiation or maturation is still unknown. Unfortunately, there is no information on LH levels in neonatally ovariectomized female monkeys.

6.4f The different LH responses to gonadectomy of males and females before puberty

Apart from the neonatal period, LH levels in castrated male marmosets rose between 160-230 days of age (Figure 6.14) just prior to the rise of testosterone levels in normal animals at about 250 days (taken as marking the onset of puberty in the male). In contrast, LH levels in ovariectomized females (Figure 6.16) did not rise until after 350 days of age (around the time of first ovulation). These results reflect some preliminary findings when marmosets of 180-250 days of age were injected intravenously with 2 μg. of LH-RH (J.K. Hodges and D.H. Abbott, unpublished work). Some of the males were capable of producing a maximal LH response (82-95 ng/ml) in 30 minutes, similar to the response of adult males (approximately 80-120 ng/ml; Hodges, 1977), whereas the females did not respond (24-34 ng/ml). Older females (between 330 and 450 days of age) showed a maximal response (78-115 ng/ml). This difference in age suggests that negative feedback is more completely developed in the immature male marmoset than in the female, and that males and females differ in the rate of maturation of their hypothalamic-pituitary unit. Gonadectomized adult marmosets showed the elevated LH castration response three days after gonad removal (Hodges, 1977, 1978). The difference between the sexes in their LH responses to neonatal gonadectomy implies some prenatal organization of the hypothalamic-pituitary negative feedback response. However, this contrasts with the work on the positive feedback response

in this monkey, where no sexual dimorphism was found (Hodges, 1977 ; Hodges and Hearn, 1978). The exact nature of differentiation at the hypothalamic-pituitary level still remains unresolved.

In the light of the above results it is difficult to view the appearance of high levels of plasma oestradiol in female marmosets of 200 days of age and over as an indication of the onset of puberty (Chapter 4). However, high levels of ovarian oestradiol secretion may be infrequent until about 350 days of age and gonadectomy may not have a sufficiently large effect on LH secretion until that age. The sensitivity limit of the LH assay is 20 ng/ml (Hodges, 1978), and this may not be low enough to detect changes in plasma LH concentrations in immature ovariectomized females. In contrast, plasma testosterone concentrations in the male are frequently pulsing up to 30-50 ng/ml after 250 days of age (Chapter 4) and gonadectomy might be expected to significantly elevate plasma LH levels.

A similar difference between the sexes was found in sheep where LH levels rose promptly in males castrated after birth, but did not rise until after six weeks of age in neonatally ovariectomized females (Foster, 1974). In rhesus monkeys, however, the difference between the sexes was reversed. In ovariectomized immature females, LH levels rose within 90 days of removing the gonads, whereas most of their castrated male counterparts took over 200 days to show the same response (Dierschke, Karsch, Weick, Weiss, Hotchkiss and Knobil, 1974 a). FSH levels followed a similar pattern to LH in both sexes. Apparently these high levels of gonadotrophins occurred before puberty (in contrast to R.W. Goy's findings above), but no precise data was given in this respect. In the human, while there is no evidence to substantiate whether or not there is a sex difference in negative feedback

in immature individuals, there is evidence that such a system operates in prepubertal children (e.g. Laron and Zilka, 1969; Penny, Guyda, Baghdassarian, Johanson and Blizzard, 1970). However, in the absence of functional gonads (e.g. Turner's syndrome), the most dramatic rise in gonadotrophins still occurs at puberty (Grumbach, Roth, Kaplan and Kelch, 1974b).

6.5 Summary

(1) This chapter contains the first report of an effect of neonatal androgenization on behaviour in a primate. Female marmosets androgenized neonatally displayed enhanced masculine rough-and-tumble play and sexual behaviour without the loss of their feminine behaviour. There was no effect of neonatal testosterone on aggression, social status, body size, attainment of puberty (the capacity to ovulate), or fertility of females. However, the clitoris of each animal was permanently hypertrophied.

(2) Other primates may also have a period of neonatal behavioural differentiation similar to the marmoset. Newborn male rhesus and pig-tailed macaques and humans also have a neonatal rise in testosterone similar to the marmoset, but the function of this testosterone remains to be determined. At present, sexual differentiation in higher primates is thought to be restricted to fetal life.

(3) Because castration of males after birth elevated circulating LH levels in the neonatal period, the testosterone secreted at this time would appear to be biologically active, at least at the level of the hypothalamus and/or pituitary.

(4) The sexes differed in their LH responses to neonatal gonadectomy, implying that negative feedback was better developed in the immature male marmoset.

CHAPTER 7 GENERAL DISCUSSION

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7.1 General discussion

The purpose of this study was to investigate sexual development and differentiation in the marmoset monkey. Since it is the first such study in a New World primate, most of the initial work was limited to providing accurate basic information on normal sexual development. The later studies utilised this data to provide some of the first information on how social dominance inhibits marmoset reproduction, and how neonatal testosterone may play a role in behavioural differentiation. These findings have established that the marmoset can be used as a primate model for research into sexual development and differentiation, that may be applied to the human in the future, and have shown that the marmoset is a practical alternative to the much-used rhesus macaque. The marmoset is particularly well suited for studies of sexual behaviour. It is monogamous, and therefore perhaps closer to the human norm in the Western world today (Short, 1976a; Kleiman, 1977) than the polygamous macaques, it generates its own control animal (88% of pregnancies result in multiple births; Lunn and Hearn, 1978), and it is small so it does not need large cages and ranging space (Hearn et al, 1975). Hence, while the marmoset is not closely related to the human in taxanomic terms, it is as suitable a model as Old World primates for sexual research.

However, before any specific conclusions are drawn from this study, it would be pertinent to summarise and discuss some of the major points raised in the results chapters. To begin with, both sexes in any one age group look alike because there is no difference in bodily size and appearance, apart from the external genitalia and and the enlarged mammary glands of multiparous females (normally well hidden in the thick fur under the arms). This lack of dimorphism is

typical of a monogamous species (Short, 1977; Clutton-Brock et al, 1977) and the monogamous social system of the common marmoset in the laboratory has been further supported by Stevenson's field studies (in press, a). These monkeys also show surprisingly little variation in their patterns of growth, especially in knee-to-heel length (Chapter 3), and this might be useful in studies of growth abnormalities.

The marmoset is a rapid developer in comparison to the rhesus monkey, chimpanzee and human. Marmosets mature within 18 months to two years and are hence well suited for studies on puberty. The age at puberty (when the animals are capable of reproducing) and the age at sexual maturity (when the animals most frequently reproduce for the first time) are clearly separated. Whether this is because of inexperience in sexual behaviour or because of female adolescent sterility, as found in other primates (rhesus: Dierschke et al, 1974a; chimpanzee: Tutin, 1976; human: Brown et al, in press), remains to be seen.

In comparison with polygamous species such as macaques and chimpanzees, developing marmosets are far less precocious in their display of sexual behaviour (see Chapter 4 for references). This might reflect differences between the restrictive practices of the marmoset's monogamous breeding system, where most of the reproductive behaviour is limited to the breeding pair, and the promiscuous behaviour observed with the polygamous macaques and chimpanzees. Sexual maturation of young in some monogamous mammals (e.g. dwarf mongooses (Viverridae) and jackals and coyotes (Canidae) occurs later than in related non-monogamous forms (e.g. the racoon (Procyon lotor: Procyonidae) and the mongoose (Herpestes auropunctatus: Viverridae) (Kleiman, 1977).

Marmosets born in captivity are particular about their sexual partners and randomly selected pairs have not been successful in breeding (e.g. Chapter 4; Kingston, 1975). Hence peer groups of monkeys (unrelated animals) are now regularly established to give the animals some choice of mate. These have proved particularly successful in producing offspring. In carrying and caring for infants, the male marmoset is noted for his large degree of parental investment (Kleiman, 1977). He does everything but lactate. Nonetheless, maternal age and experience are more important than paternal in rearing offspring (Chapter 4; Epple, 1978; Ingram, in press). Young females that initially fail to raise their offspring with one partner, usually fare no better if given an experienced male partner. Whether this deficiency is physiological (i.e. an inability to lactate properly) or behavioural (i.e. discarding or attacking the young soon after birth) remains to be determined, but it does seem that bad young mothers remain so (e.g. Table 4.5, Chapter 4). However, it is still too early to compare this data with parallel observations in the human, i.e. the 'battered baby' syndrome.

The captive marmoset breeding group obeys one cardinal rule. Only the dominant female breeds. The dominant male is also thought to be the only male to reproduce, but the information on this is not as conclusive as that for the dominant female. In family groups (related animals) only the mother and father breed even though sexually mature sons and daughters can ejaculate spermatozoa and ovulate, (see Chapter 5). However, mating is almost exclusively limited to the parents, implying some form of behavioural inhibition restricting the mature offspring, e.g. an incest taboo. Such taboos are common in human societies (reviewed by Ford and Beach, 1952) and a mother-son incest taboo

is perhapspresent in rhesus monkeys (Missakian, 1973). In monogamous species, inhibition of reproduction after the age of sexual maturity is common in juveniles remaining with their parents (Kleiman, 1977). This is well documented for marmosets and tamarins (Epple, 1967, 1978; Rothe, 1975), the dwarf mongoose (Rasa, 1973) and the wolf (Mech, 1970). The pattern of juveniles and sub-adults caring for subsequent litters of their parents while their own reproduction is inhibited has been referred to as "parental manipulation of progeny" (Alexander, 1974). Such "manipulation" would improve the reproductive output of the parents at the expense of their offspring, at least for a time. But, the offspring would benefit in the prolongation of parental protection through continued association with the family unit and the aquisition of experience in rearing young. As free-living groups of marmosets have a similar social structure to captive groups (Epple, 1978; Stevenson, in press, a; A.S. Neto, personal communication) any suppression of fertility in mature offspring would help prevent inbreeding and enhance any movement away from the family group into other groups or sub-groups, where such suppression may be removed. In laboratory peer groups of marmosets (unrelated animals) social status is established by fighting within 2-3 days of group formation (Chapter 5; Epple, 1975; Rothe, 1975). Aggression is predominantly between animals of the same sex, giving the impression of two separate hierarchies commonly observed in monogamous species including other marmosets and tamarins (Epple, 1975; Rothe, 1975), wolves (Zimen, 1976), and dwarf mongooses (Rasa, 1973). The dominant male and female marmoset form a pair bond (denoted by their associative behaviour), and after 2-3 days following formation of the peer group, aggressive interactions settle down to a low level and individual rank is

difficult to determine (similar to the situation perpetually found in family groups). Nevertheless, the dominant pair are still obvious because of their association (e.g. male following female, etc.). Only the dominant female breeds because the other females stop ovulating. This infertility is possibly due to the higher prolactin levels found in subordinates; high prolactin levels are known to inhibit female reproductive function in talapoin monkeys (Bowman et al, 1978; Keverne, in press). The syndrome of hyperprolactinaemia-amenorrhea is also an anovulatory condition in women (Besser et al, 1972). The available evidence (mainly from work on rodents) suggests two possible inhibitory mechanisms where elevated prolactin levels are involved (A.S. McNeilly, personal communication). In the first place, high prolactin levels may be the primary causal agent. Elevated circulating levels of prolactin are known to stimulate the turnover of dopamine (a neurotransmitter which acts in the negative feedback control of prolactin in the hypothalamus; Hökfelt and Fuxe, 1971; Olson, Fuxe and Hökfelt, 1972) and to suppress circulating gonadotrophin levels (McNeilly et al, 1978). Increased dopamine turnover is also known to suppress gonadotrophin secretion (Fuxe, Hökfelt, Agnati, Löfström, Everitt, Johansson, Wuttke and Goldstein, 1976), presumably by reducing the hypothalamic output of LH-RH. The reduced secretion of LH-RH, and hence reduced gonadotrophin output, would lead to a much reduced gonadotrophin response to steroid hormones secreted from the gonads and effectively resemble an increase in sensitivity to negative feedback (perhaps similar to that of the pre-pubertal state; see Grumbach et al (1974a) for reviews). Furthermore, with the example of subordinate female marmosets, reduced stimulation of the ovaries would lead to reduced ovarian steroid production (and hence perhaps reduced follicular development). Indeed, Lunn (in press) has found that

urinary oestrogen excretion from subordinate females is typical of ovariectomized animals - a totally abnormal situation for mature or post-pubertal intact females. However, for this prolactin-induced mechanism to work one must postulate an external source which can stimulate prolactin release in the first instance. This is feasible in cases such as lactational amenorrhea where the suckling stimulus of the infant can act as an external 'drive' (Baird et al, in press). But, it is difficult to postulate an equally feasible 'drive' in the case of socially-mediated reproductive inhibition. One is then drawn to postulate an alternative hypothesis. Perhaps there is a 'central inhibitor' (such as the pineal gland or the amygdala region of the brain?) which can be invoked (say by the stress of subordination) to inhibit neuro-transmitter turnover in the hypothalamus generally. This would have a two-fold affect. LH-RH output, and therefore LH and FSH output, would be reduced as before, but this time prolactin levels would be elevated as a consequence of reduced neurotransmitter turnover and would be purely a secondary effect. This hypothesis, however, remains to be proven, but Peet and Lincoln (1977) have shown that in the sheep, hypothalamic suppression (or at least suppression of LH-RH release) can be obtained using a general anaesthetic (or "central nervous system depressant") such as Immobilon. The pituitary, nonetheless, could be stimulated to produce LH if exogenous LH-RH was given. The whole arguement for reproductive inhibition in subordinate female marmosets is therefore based on an effect at the hypothalamus, but it could be that only the ovary is directly affected. This is unlikely because increased circulating levels of LH would be expected in subordinate females as a negative feedback response to the reduced

steroid production from the suppressed ovaries, and this is not found (J.K. Hodges, personal communication; D.H. Abbott, unpublished work).

Returning to the laboratory peer groups of marmosets, the dominant male might not be the only male to breed because he does not completely inhibit other males from copulating with the dominant female. Subordinate males do not suffer from the chronic inhibition of reproductive function found in the subordinate females. Although it has yet to be studied in marmosets, the dominant male may deter subordinates sufficiently when the dominant female is about to ovulate and hence ensure his exclusive breeding rite. Therefore, whatever the composition of the laboratory group (i.e. related and/or unrelated animals) apparently only one male and female reproduce and behavioural and/or physiological mechanisms operate to maintain this monogamous status quo. These mechanisms may just be more extreme versions of those already operating in polygamous primate species (e.g. rhesus monkeys (females): Drickamer, 1974; Gelada baboons (females): Dunbar and Dunbar, 1977; yellow baboons (males): Hausfater, 1975).

The study then culminated in the first report of an effect of neonatal testosterone on behaviour in a primate. Female marmosets androgenized neonatally (when males normally experience high testosterone levels) showed enhanced masculine rough-and-tumble play and sexual behaviour in later life, long after the testosterone implants had been removed. Apparently, the neonatal testosterone had had some behavioural organising effect (but see Chapter 6 for other possibilities). Previously, behavioural differentiation in primates was thought to be restricted to fetal life because of successful masculinization of female rhesus monkeys and humans <u>in utero</u> and the failure to achieve similar results in the neonatal period (see Chapter 1 for references). However, the

behavioural consequences of exposing genetic female primates and humans to testosterone in the immediate postnatal period has not been sufficiently explored and these primates and Man may also have a hitherto unknown period of neonatal behavioural differentiation similar to the marmoset. This, of course, presumes that neonatal testosterone in the male marmoset performs a similar function to the testosterone implanted into females. In the light of such results, perhaps care should be taken to avoid any hormonal treatment of newborn baby girls and boys before more is known about this period of development, particularly in the treatment of premature babies. In the past, premature babies have been given testosterone to improve their chances of survival, but little or no attention was paid to the subsequent behaviour of female patients (e.g. Polishuk and Anteby, 1971). Furthermore, until recently (R.V. Short, personal communication), testosterone was given to women in labour to relieve "after pains" and/or suppress lactation (The Pharmaceutical Society of G.B., 1968; Todd, 1967). It had not been recognized that this might represent a potential hazard to the normal development of a female infant and oestrogen and progesterone are now preferred (Wade, 1977). Androgens or oestrogens are also given to suppress established lactation. Even this may represent a hazard to normal female development by transmitting these steroids in milk produced before suppression is complete. However, it is not yet known whether sufficient androgen or oestrogen could get through into the milk and subsequently be absorbed from the intestinal tract of the newborn to have any appreciable adverse effect.

Nevertheless, while neonatal androgenization may have implications for the human and other primates, the marmoset may be unique in its

hormonal method of imprinting sexual differentiation because of its unusual embryology (see Chapter 1 for references). Dizygotic twinning is very common and yet despite the early development of a shared placental circulation and haematopoietic chimerism, females born co-twin to a male are totally unaffected (Wislocki, 1939; Benirschke and Layton, 1969). This is quite unlike the situation in animals such as cattle, sheep, goats and possibly pigs where females born co-twin to a male are sterile freemartins (Marcum, 1974). However, none of the mechanisms proposed for initiating the freemartin condition are satisfactory and certainly none of them can explain the lack of freemartinism in XX/XY chimeric female marmosets (see Chapter 1). The obvious explanation for the lack of freemartin marmosets is that none of the 'male inducing substances' escape into the general circulation and have a very localized effect on the male co-twin, e.g. testicular androgens, Müllerian inhibiting hormone, H-Y antigen, testicular inductor cells or substance. The H-Y antigen has been shown to be crucial for the expression of masculine characteristics and increasing experimental evidence has suggested that it is a plasma membrane protein and the testis-determining gene product (Wachtel, Ohno, Koo and Boyse, 1975; Ohno, 1976 review; Ghosh, Shah and Gharpure, 1978) located on the Y-chromosome (Bennett, Mathieson, Sheid, Yanagisawa, Boyse, Wachtel and Cattanach, 1977). However, all the evidence cited about the H-Y antigen is circumstantial, based on correlations between gonadal morphology and H-Y antigen status in normal and intersex individuals. The H-Y antigen may not also be the sole male-determining factor on the Y-chromosome since it does not seem to account for germ cell sex. Germ cell sex may be under an entirely independent genetic control mechanism because immature male germ cells are probably the

only cells in the human male body that are H-Y negative. Apparently they only become positive in the late spermatocyte stage (Zenzes, Muller, Aschmoneit and Wolf, in press). Nonetheless, if any potentially masculinizing factors do leak out into the general circulation of a male marmoset fetus, they may be prevented from reaching the co-twin female. For example, the marmoset placenta, like the bovine placenta (Pierrepoint et al, 1969), can aromatize androgens into oestrogens (Ryan, Benirschke and Smith, 1961) and so perhaps negates any masculinizing influence androgens from the male might have on the external genitalia of a co-twin female. However, there appears to be as much circulating androgen in female fetuses as in their male co-twins (P.L. Chambers, personal communication). In this respect it is interesting to note that female mice are partly masculinized before birth if they are located next to a male in the uterus (Gandelman, Vom Saal and Reinisch, 1977). These effects are possibly mediated by localized diffusion of androgen between adjacent amniotic membranes. There are also no known mechanisms to prevent other factors from crossing between fetal circulations, such as the Müllerian inhibitor. Perhaps the fetal female marmoset is relatively insensitive (in comparison to the male) to any of the virilizing factors mentioned above. It may be that only XY cells have the capacity to bind and/or react to such factors as Müllerian inhibiting hormone or the H-Y antigen and XX cells cannot respond, i.e. marmoset cells with a Y-chromosome may have a differently composed or shaped cell membrane to cells without a Y-chromosome, and perhaps an alteration in the composition of membrane receptor sites (which bind the circulating factors) may give the XX cells effective 'immunity' from all of the above factors. Ohno (1976) has shown that the reason why XX female fetuses of other species readily masculinize in response to

exogenously administered androgen may be because they are equipped with the same amount of specific nuclear-cytosol androgen receptor protein as XY cells. Such a receptor protein must be coded on either the X- or an autosomal chromosome. However, if during evolution and the development of twinning (Leutenegger, 1973) the marmoset has managed to translocate this genetic coding to the Y-chromosome (and perhaps the receptor proteins that bind all the masculinizing factors and the Mullerian inhibitor are positioned together on one part of a chromosome) then only the XY cells of the male embryo will participate in masculine development and the XX cells of the female will be unaffected. This 'insensitivity' of the female may just be limited to fetal life or it may just be exhibited to a certain degree. In the case of the latter, the large amounts of testosterone given to androgenize neonatal females could have overcome the 'insensitivity barrier'. It will be difficult to test such an hypothesis but it would be fascinating to see if purified human H-Y antigen could change marmoset fetal ovaries into testes <u>in vitro</u> in the same way as it transforms bovine fetal ovaries into testes in five days (Ohno, Nagai, Ciccarese and Iwata, in press; Nagai, Ciccarese and Ohno, in press).

The answer as to how male and female marmosets differentiate separately therefore still remains unattainable. However, because neonatal androgenization of female behaviour was effective, the marmoset may have delayed some facets of sexual differentiation until after birth, such as the brain centres controlling behaviour. Nonetheless, this explanation is too simple because while neonatal testosterone enhanced the masculine behaviour of female marmosets to a certain degree, it did not completely masculinize their behaviour and it did not abolish their feminine sexual responses. Their feminine

responses could of course be solely due to the lack of adult testosterone levels in such androgenized females and the presence of their own ovarian hormones. Furthermore, males castrated soon after birth showed some components of masculine sexual behaviour (though no mounting or pelvic thrusting), and showed little or no feminine sexual behaviour, suggesting that some behavioural organization of their brain had taken place, presumably under prenatal hormonal control. The deficits in their masculine behaviour could, of course, be due to the lack of testicular testosterone postnatally. These findings with the castrated males suggest that the marmoset may undergo both pre- and post-natal behavioural differentiation and that female fetuses are in some way 'protected' from any 'masculinising influences' (e.g. high concentrations of testosterone) carried through the joint circulation with their male co-twins (as previously mentioned). Whether behavioural differentiation in the marmoset is purely postnatal or pre- and post-natal remains to be determined.

Whatever the explanation surrounding sexual differentiation in the marmoset, the fact still remains that neonatal androgen can influence the subsequent performance of adult and post-pubertal genetic females by enhancing masculine patterns of behaviour. The implication that differentiation of the primate brain may span more stages of development than is currently considered cannot be ignored.

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APPENDIX A

DEFINITIONS OF MARMOSET BEHAVIOUR RECORDED ON THE COMPUTERISED SYSTEM BUT NOT ANALYSED IN THIS STUDY

"GROOMING":

self-groom: where an animal groomed itself using its hands. Back-scratching and ear-scratching using the feet was also included.

lick or groom genitals: where an animal licked or groomed the genitals of another or itself.

sniff genitals: when an animal sniffed its own genitals or those of another (usually on approaching a stranger).

body sniff: sniffing the head or body of another animal.

"AVOIDANCE"

avoid: quickly moving away from the approach (to within approximately 30 cm.) of another animal.

hand: pushing another animal away with the flat of the hand(s). However, this is also used in play and in jostling positions while huddling.

"INFANTS":

climb on and climb off: when infants climb on or off the backs of their older siblings or parents without any previous intimidation.

take: when infants are actively encouraged by their parents or older siblings to climb on to their backs.

reject: when parents or older siblings actively force infants off their backs.

"FOOD OR OBJECTS":

food: animals eating the food supplied

chew object: gnawing or chewing objects other than the food supplied, i.e. pieces of bark, wood shavings or twigs.

food or object steal: stealing food or an object from another animal.

"SCENT MARKING":

scent mark (anal): when animals rub their pudendal pad against objects (such as a branch) or cagemates, presumably depositing scent from the pad's secretory glands (e.g. Sutcliffe and Poole, 1978).